The Biological Assay of Chorionic Gonadotrophin in Relation to Problems in Clinical Medicine.

by

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INTRODUCTION

The science of female endocrinology is, as yet, a relatively young one, but probably in no other field of medicine has there accumulated in so short a time such a vast and rapidly expanding literature embracing both clinical and experimental aspects of a subject, the true foundations of which were laid barely two decades ago and which, prior to this, had been shrouded by a haze of mystery and empiricism. Evolution has indeed been so rapid that it has been well-nigh impossible to digest and assimilate properly the rich diet of fundamental discoveries scattered in many publications and in numerous languages during these years.

It must not be assumed, however, that the research, entailing such an adventurous conquest of new territory, has pursued an untrammelled course. The road to discovery has been beset by many pitfalls. Attractive hypotheses have been advanced, too hastily accepted and as hastily rejected. Too frequently the wide gap existing between the laboratory animal and the human has not been appreciated sufficiently and/

and speculative and ill-founded concepts have held sway for limited periods among evidence which is never static but continually grows and fluctuates.

Medicine owes much to the pioneer work of Smith (1926) and of Smith and Engle (1927) who, developing for the first time a satisfactory technique of hypophysectomy in the rat showed that total ablation of the anterior lobe of the pituitary caused profound involution and atrophy of the gonads and accessory genitalia, that this phenomenon could be prevented by the implantation into the hypophysectomised animal of anterior lobe extracts and that in the immature intact animal these same implants could induce precocious sexual maturity. Thus the great importance of the anterior pituitary in the initiation, control and maintenance of gonadal function was first demonstrated.

The discovery of Aschheim and Zondek (1927) that the urine of pregnant women contained a gonad-stimulating substance which, when injected into immature female mice, produced follicular maturation, luteinisation and haemorrhage into the ovarian stroma, not only gave the scientific world its first reliable test for the diagnosis of pregnancy, but caused/

caused the very meagre stream of contributions which prior to that date, had been devoted to gonadotrophic activity soon to be swollen to a veritable flood.

The years immediately following these landmarks in the study of gonadotrophins were clouded by controversy and conflict of views, both as regards nomenclature and also in the interpretation of findings obtained experimentally. In retrospect it may appear almost paradoxical that the conclusions drawn by Aschheim and Zondek were based on a misconception. These investigators believed that the gonad-stimulating effect produced by pregnancy urine was due to hormones released from the anterior lobe of the pituitary and they described two hormones in this connection. These were prolan A corresponding to Follicle Stimulating Hormone (F.S.H.) the main effect of which in the female rat, or mouse, is to cause growth and development of ovarian follicles, and in the male to stimulate spermatogenesis, and prolan B corresponding to Luteinising Hormone (L.H.), its chief action being the formation of corpora lutea in the female and stimulation of the interstitial cells of the testis in the male. They also stated, and in this they were correct, that the effect of pregnancy urine/

urine was predominantly luteinising in nature.

Engle (1929) was the first to question the pituitary origin of the gonadotrophin of pregnancy urine and since then a wealth of experimental evidence has been presented by numerous investigators that the main source of the gonadotrophic principle which floods the body during pregnancy is the placenta and not the pituitary as previously believed. (Collip, 1930:1934:1935; Collip, Seyle and Thomson, 1933; Hamburger, 1933; Deansely, 1935). Indeed, it has been frequently shown that during pregnancy, the gonadotrophic hormone content of the pituitary is very low. This has been demonstrated in the human by Phillip (1930), and by Zondek (1931); in the cow by Nalbandov and Casida (1940), and in the horse by Hellbaum (1935).

Important differences in biological effects exist between the action of gonadotrophic hormone extracts obtained from the pituitary, the urine of males, of normal, menopausal and post menopausal women on the one hand, and those obtained from the placenta and the urine of pregnant women on the other (Evans, Meyer and Simpson, 1933; Evans, 1933; Evans, Gustus and Simpson, 1933). For instance, pituitary extracts when tested in immature rats cause/

cause marked increase in ovarian weight provided sufficient extract is given, whereas those from pregnancy urine and placenta produce only a limited increase in ovarian weight no matter how much is given. (Orbin and Watrin, 1929; Engle, 1933; Schockaert, 1933; Fluhmann, 1933, 1934). Superovulation which can easily be induced in the mature rat by administration of pituitary hormones cannot be duplicated by chorionic gonadotrophin (C.G.), (Evans and Simpson, 1940) but the latter, if given over a long period, is much more likely to produce abnormal ovaries with luteinised follicles and imprisoned ova. In the immature rat, according to Seyle, Collip and Thomson, (1933) before follicles are present thecal luteinisation is much more marked with C.G. than with pituitary hormones.

Perhaps the most cogent evidence for the placental origin of the hormone, however, is the dramatic observation that the gonadotrophic hormone is produced in vitro in placental tissue culture, (Hein, 1926; Sannicandro, 1934; Gey and Jones, 1938). Gey and Jones (1943) on the basis of their work on tissue culture with five three month old placentae, two placentae from ectopic pregnancies, two full term placentae and three hydatidiform moles concluded that the/

the placental cells produced C.G. and that this property was specially marked in the Langhans cells which may retain this capacity for up to six months. In a recent paper Stewart, Sano and Montgomery (1948), have produced results in support of Gey and his co-workers. They showed that human placental cells of approximately three months of age grown in continuous tissue culture in vitro produced gonadotrophin and that there was a distinct correlation between the growth of the Langhans cells and gonadotrophic hormone production. In addition to producing gonadotrophin the placenta also is believed to elaborate oestrogen,(Fellner, 1922; Waldstein, 1929; Dempsey and Wislocki, 1944 and 1946) and progesterone (McGinty and McCullough, 1936; Ebrhardt, 1936). Progress has indeed been made since Strachan, (1925) declared "beyond the accepted fact that the placenta is the organ of alimentary and excrementitious exchange between the mother and the foetus we cannot go a single step forward without falling into a quagmire of dispute and doubt."

It is quite evident that the above experimental work has been extensive enough to establish and define the two great groups of gonadotrophins - those of hypophyseal origin and those of extra hypophyseal origin. The present thesis deals with the hormone/

hormone formerly called pregnancy prolan or anterior pituitary-like substance (A.P.L.) but now known as chorionic gonadotrophin (C.G.). The equine hormone from the serum of pregnant mares (P.M.S.) was first demonstrated by Cole and Hart (1930). It is predominantly follicle-stimulating in action but will not be considered here as it is of little importance in human medicine apart from the therapeutic field.

Work on C.G. has been directed into many and varied channels. The investigation of techniques of assay by biological methods forms an important part of the subject and will be discussed in some detail below. Hand in hand with improvement in methods of extraction of C.G. from blood and urine, knowledge of the chemistry of the hormone has advanced. The qualitative aspect of the problem has been exploited by the investigation of methods of pregnancy diagnosis based on the detection in the urine of certain quantities of gonadotrophin produced during pregnancy and excreted by the kidneys. A very large number of pregnancy tests have been proposed but only the Aschheim-Zondek test, the rabbit ovulation test, (Friedman and Lapham, 1931), and the test employing the female South African clawed toad Xenopus laevis, (Hogben, Charles and Slome, 1931) have stood the test/

test of time.

Recently, quantitative considerations have bulked largely in the mind of investigators. Much work has been carried out on the concentration of C.G. in blood and urine in normal pregnancy, (Browne and Venning, 1939; Jones, Delfs and Stran, 1944; Loraine, 1949). In pathological conditions too, interest in the excretion of C.G. is rapidly awakening and a vast new field may soon be opened up. It is already known that the excretion of C.G. in hydatidiform mole and chorion-epithelioma is usually greater than in normal pregnancy, (Fels, 1929;Rossler, 1929; Boycott and Smiles, 1934; Crew, 1936; Zondek, 1937; Hamburger, 1943). The work of White (1937), White, Titus, Joslin and Hunt (1939), and White and Hunt (1943) on pregnancy complicated by diabetes, of Smith and Smith (1939;1948) and Watts and Adair (1943) on pre-eclampsia and eclampsia and of Anselmino and Hoffmann (1936); Rakoff (1940) and Schoeneck (1942) on hyperemesis gravidarum forms a fascinating new chapter in endocrinological progress imbued with tremendous possibilities both from the diagnostic and therapeutic points of view.

A great variety of methods have been proposed for the biological assay of human chorionic gonadotrophin. The test animals have usually been rats/

rats or mice initially lacking gonadotrophic hormone either from youth or hypophysectomy. The index of response has varied greatly (Thayer, 1946). The effects can be divided into two great groups - primary, where the organs inspected are the gonads themselves, i.e., ovaries in the female animal and testes in the male, and secondary, which are due to the liberation from the gonads of oestrogen or androgen.

In the primary group, which should theoretically give more reliable results, observations have been based on corpora lutea (Aschheim and Zondek, 1927; Kennedy, 1933; Siegler, 1939; Smith and Smith, 1939; 1948; Emmens, 1939; White, 1943); ovarian weight (Wallen-Lawrence and Van Dyke, 1931; Evans, Meyer and Simpson, 1929;1931;1933; Evans, Kohl and Wonders, 1937); ovarian hyperaemia in rats (Zondek and Sulman, 1945; Zondek, Sulman and Black, 1945); rabbit ovulation, (Friedman and Lapham, 1931; Schoeneck, 1942) and Xenopus (Hogben, Charles and Slome, 1931; Crew, 1939); Landgrebe, 1948).

In the secondary group, use has been made of uterine weight (Emmens, 1939; Sealey and Sondern, 1940; Jones et al, 1944; Dorfman, Rubin and Miller, 1946; Dorfman and Rubin, 1947); vaginal smears (Wallen-Lawrence and Van Dyke, 1931; Browne and Venning, 1936(a); 1936(b)/

1936(b); 1936(c); 1938; 1939; Venning, 1948); vaginal smears in B deficient rats (Heard and Winton, 1939; Heard, 1941); seminal vesicles (Korenchevsky, Dennison and Levy Simpson, 1935; Bischoff, 1936; Deansely,1935; Sealy and Sondern, 1940; D'Amour and D'Amour, 1940; Watts and Adair, 1944) and prostate (Loraine, 1949).

Many of these methods have considerable disadvantages such as insensitivity (Xenopus), variability in sensitivity with the rat colony used (ovarian hyperaemia) and inherent inaccuracy (corpora lutea). Hamburger and Pedersen-Bjergaard (1937) concluded that the rat ovary was much more inaccurate than the uterus or vaginal smears. In a recent review, Emmens (1939) concluded that the vaginal smear method was the most accurate one and that involving the uterine weights, least accurate.

In the interpretation of results much confusion has arisen through the adoption by workers of a multitude of arbitrary "rat units" and "mouse units" varying in each laboratory and based on methods involving variations in the degree of glandular response of the animal as determined by changes in the weight of the organ or by histological examination. The introduction in 1939 of a standard preparation of chorionic/

chorionic gonadotrophin was a great step forward. The definition is:- "the specific gonadotrophic activity of 0.1mg.(100γ) of the standard powder shall be the international unit for recording activities of all gonadotrophic hormone preparations of human pregnancy urine but only of such." (Bull. League of Nations Supplement, 1939). Using the standard preparation, results from different laboratories are directly comparable no matter how many types of test are used. The method described below is based on the use of this international standard.

Methods of extraction of gonadotrophins from the blood and urine in pregnancy are also very numerous. Precipitation by alcohol was the original method used by Zondek and Aschheim (1929) and also by Fluhmann (1932); Shapiro and Zwarenstein (1934, 1935); Taylor and Scadron (1939); Watts and Adair (1944). Acetone precipitation has been favoured by Frank and Salmon (1935); Crew (1939) and Loraine (1949). Katzman and Doisy (1934) evolved a rather laborious method entailing the adsorption of the hormone on benzoic acid and elution with dilute NaOH. They claimed thereby to have produced a very pure preparation. This method was subsequently used by Fevold and Hisaw (1936) and by Heard and Winton (1939). Tungstic/

Tungstic acid precipitation was also suggested by Katzman and Doisy (1932-33), but was soon abandoned as the quantitative recovery of the hormone was found to be poor. Fevold and Hisaw (1936) obtained a fairly good recovery of the gonadotrophin from pregnancy urine by shaking it with cresol and the process of salting out with sodium sulphate was employed by Frank and Goldberger (1931). Precipitation by tannic acid was applied to pregnancy urine by Hellbaum, Fevold and Hisaw (1935) and by Levin (1941), but the method is laborious and time-consuming. Adsorption of the hormone on kaolin and elution with NaOH was introduced by Scott (1941), and since has achieved much popularity. A modification of this method involving subsequent precipitation by acetone after adsorption and elution has been applied to both pregnant and non-pregnant urine and has been found eminently satisfactory (Dekanski and Loraine, 1949). Milton (1946) claimed that adsorption of the hormone on permutit was an effective method giving good quantitative results.

Improved methods of extraction have led to an increase in knowledge of the chemistry of the gonadotrophin. It is now generally agreed that C.G. in common with the other gonadotrophic hormones, is of/

of a protein nature and belongs to the group of glycoproteins. Meyer (1938) showed that the substance contained hexosamine and that the ratio of total nitrogen to hexosamine nitrogen was indicative of the purity of the compound, the hexosamine nitrogen content increasing in relation to the total nitrogen as the extract became more purified. This was confirmed by Hartmann and Benz (1938) and by Fleischer, Schwenk and Meyer (1938).

Pure C.G. which is crystalline and electrophoretically homogeneous has recently been prepared by Claesson, Hogberg, Rosenberg and Westman (1948). The method of preparation consisted of adsorption of the hormone on benzoic acid with subsequent fractionation of the precipitate by ethanol. The next step in the procedure was precipitation by protamine to remove impurities and finally the excess protamine and any remaining impurities were separated by Reinecke salt. The crystals of C.G. were long, thin rods and needles. Electrophoretic studies on the crystalline C.G. showed a high degree of purity of the preparation and the latter was found to have constant and well defined biological activity in experimental animals. The substance had an activity of 6,000-8,000 I.U. per mg. In normal intact immature rats/

rats it caused formation of follicles and corpora lutea. In hypophysectomised animals, however, no such effects were observed in daily dosage of 1,500 I.U. of crystalline C.G. The only effect noted in these animals was some increase in the interstitial tissue in the ovary. This supports the view that the action of C.G. in producing follicles in the intact immature animal is due to synergism between the C.G. administered and F.S.H. from the animal's own pituitary. The relative non-toxicity of the pure preparation was demonstrated in the human by the fact that the substance could be given intravenously in dosage of 12,000 I.U. per day on three successive days without the production of any toxic symptoms.

In a study of C.G. therefore, there is a common meeting place for the experimental physiologist, the chemist, the endocrinologist, the obstetrician and the general physician. Only by their continued co-operation will there come a better understanding of the problems of clinical medicine based on the proper appreciation of scientific and experimental evidence, so that in the future, present ignorance will be replaced by sounder knowledge.

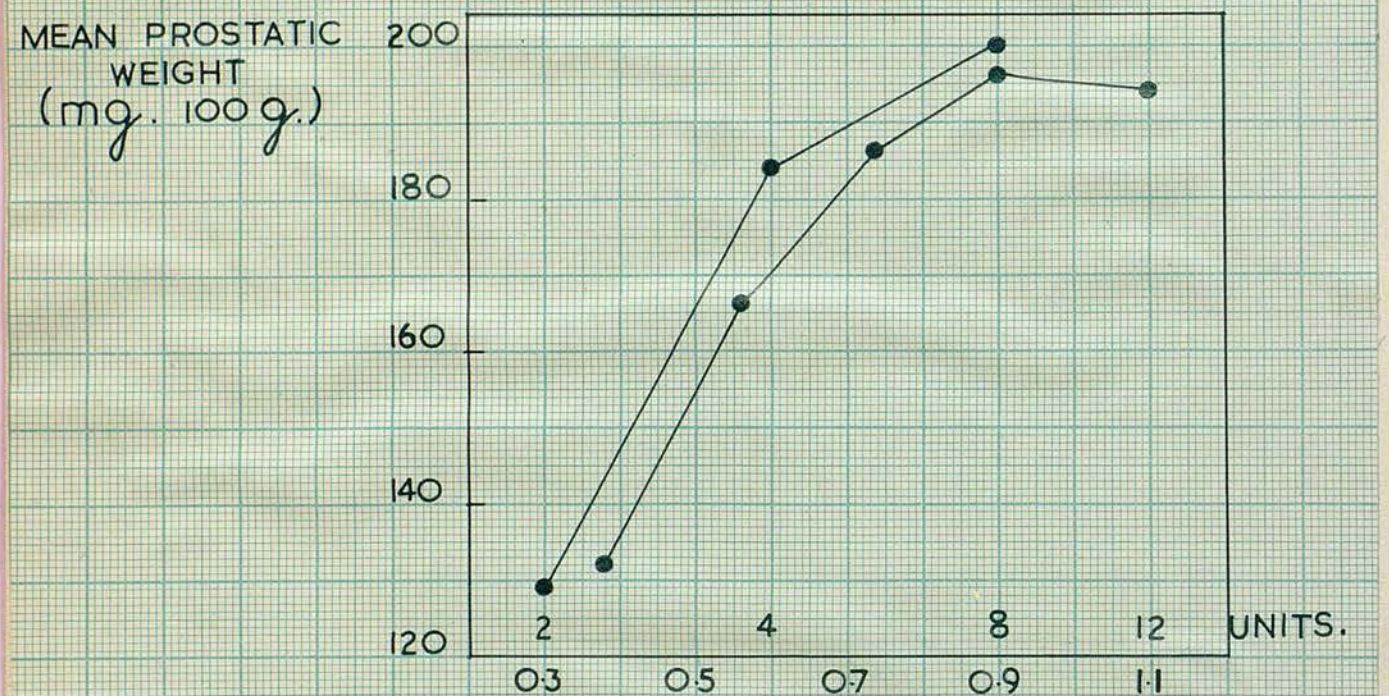

FIG I.

THE RELATION BETWEEN LOG DOSE OF STANDARD C.G. AND PROSTATIC WEIGHT IN IMMATURE RATS.

(CONTROL VALUE = 80 mg./100 g.)

METHODS.

Method of Assay.

This depends on the increase in the weight of the prostate in immature rats. Two dose-response curves, using the standard preparation of C.G. are shown in Fig.1. In subsequent assays, two doses of the standard (2 and 4 units) were used and one dose of urine chosen to give a response between those two doses of standard. In the latter part of pregnancy, i.e., after 100 days, as calculated from the last menstrual period, a total dose of 0.6 c.c. per rat spread over three injections was generally suitable. When the concentration of the hormone was high, as it is during the first 100 days of gestation in normal women, and in some diabetic and pre-eclamptic women at any stage of pregnancy, the dose was smaller. In such cases, the total dose of urine per rat should be reduced to 0.3-0.15 c.c. in three injections. It has not been found necessary to go below 0.15 c.c. per rat except in a case of suspected hydatidiform mole where doses of .015 and .03 gave responses between the two doses of standard.

The actual outline of the assay is as follows:-/

follows:- fresh untreated urine without preservative and refrigerated at 4°C. immediately after collection is employed. The portion for assay if always taken from a complete 24-hour sample of urine. The rats used have varied from 19-22 days old, wherever possible rats at 21 days being selected from a colony derived originally from the Wistar Institute. The weight of the animal appears to be more important than its actual age and rats weighing 30-45g. are suitable for the experiments, rats weighing less than 30g. initially tending to lose weight during the test. Evidence has been adduced by statistical methods that the balanced use of littermates does not markedly increase the accuracy of the experiment. Originally, 10 rats were used in each group, i.e., at least 30 rats per test. Later, in light of estimates of the error of the test the groups were reduced to 5 so that more results were obtained with the same number of rats.

The animals are injected subcutaneously for three days at approximately 24-hour intervals. They are killed at 96 hours, the prostate and seminal vesicles being carefully dissected out from the surrounding structures and fixed in Bouin's solution for 24-hours. On the fifth day, the organs/

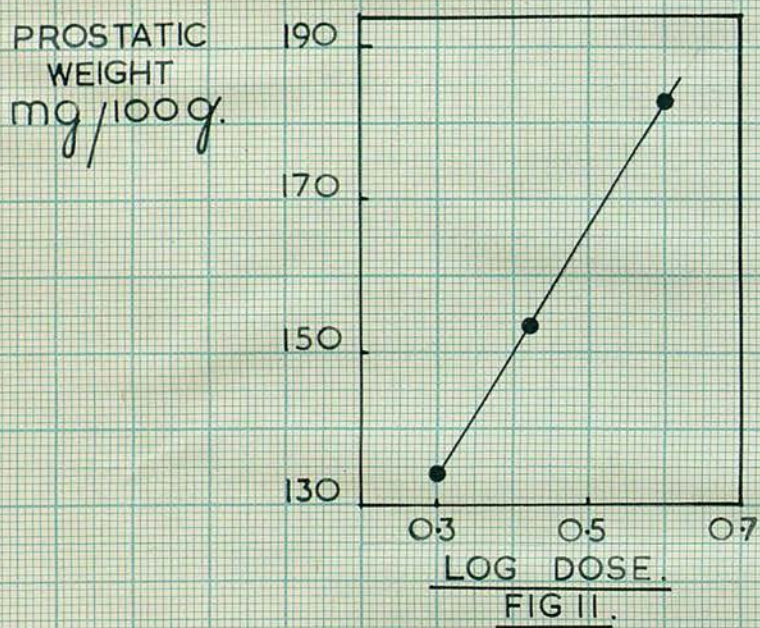

FIG II.

TYPICAL DOSE-RESPONSE CURVE.

(EXPLANATION IN TEXT.)

organs are freed from fat, the seminal vesicles discarded and the prostate dried thoroughly by fairly firm pressure between pieces of filter paper. By dissection from below, the lobes of the prostate are separated from the urethra and the whole prostate, both ventral and postero-lateral lobes, weighed on a torsion balance. The results are expressed as mg. of prostate per 100g. of rat weight as this appeared to give more consistent results than expressing them merely as mg. of prostatic weight.

A dose-effect curve is now constructed for the standard doses plotting mean weight in mg./100g. against log. dose. The mean weights for the urines being assayed are also calculated and interpolated graphically on this dose effect curve. A typical example of the calculation is shown in Fig.2 where the mean prostatic weight (mg./100g.) with 10 rats receiving each 2 I.U. was 133, and with 10 rats each receiving 4 I.U., 183. The mean weight with a total dose of 0.5 c.c. of urine per rat from the patient E.T. was 152, again 10 rats being used. The dose response curve is now constructed, plotting the log. dose of standard against mean prostatic weight (log.2=0.3: log.4=0.6). On this curve,

a/

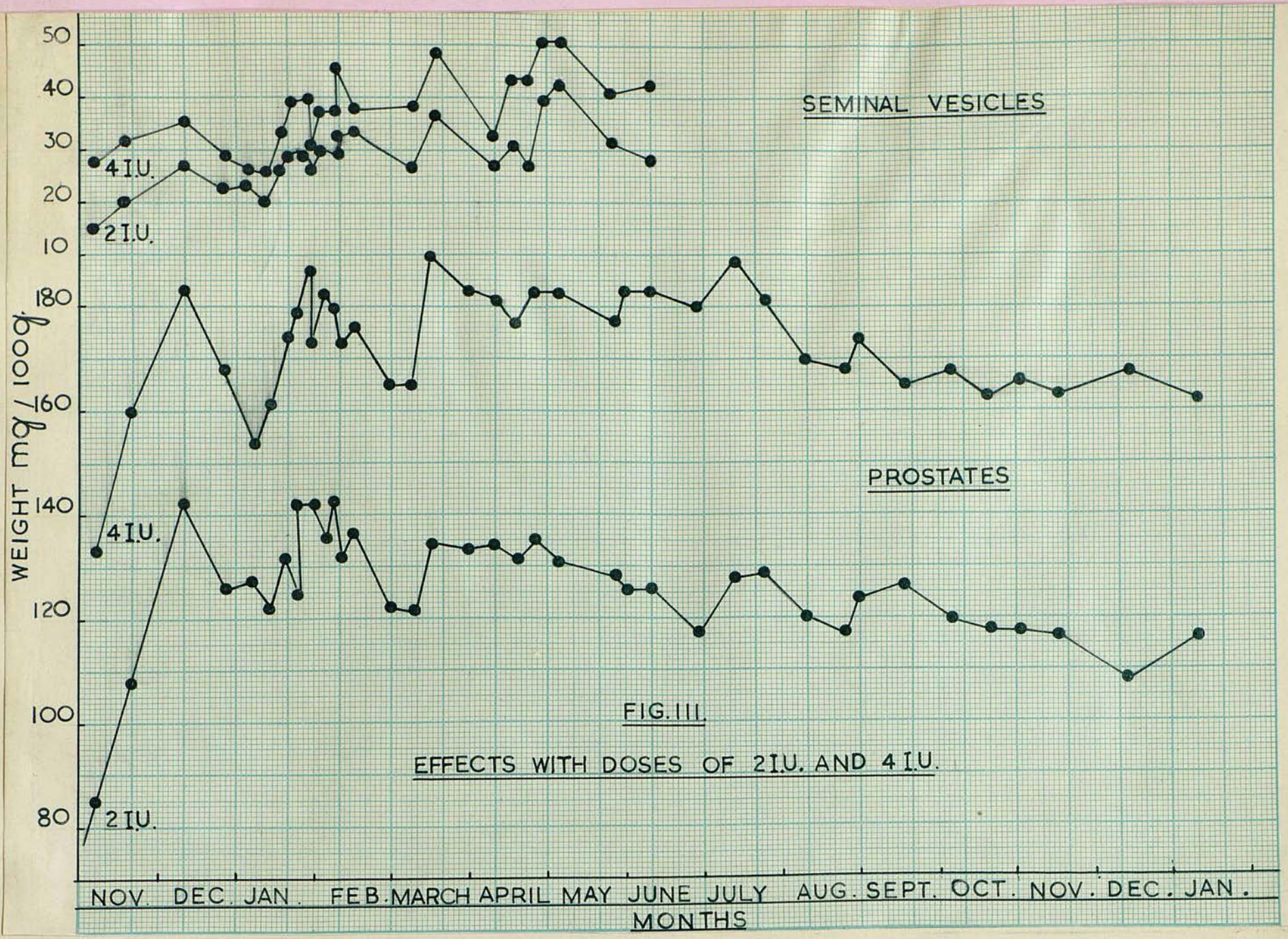

FIG. III.

EFFECTS WITH DOSES OF 2 I.U. AND 4 I.U.

a weight of 152mg. corresponds to a logarithmic value of 0.42 and the antilogarithm of this is 2.630. This is the quantity of I.U's of C.G. present in 0.5 c.c. and therefore in the total 24-hour collection, which amounted to 1,400 c.c. the quantity of C.G. was 7,364 I.U's.

It will be seen that the schedule of dosage used differs from that of Korenchevsky et al (1935), when assaying extracts made from pregnancy urine. They gave one injection per day for three days, but killed the animals at 120-hours. Watts and Adair (1944), using seminal vesicles gave two injections per day for three days and killed the rats at 96-hours. Deansely (1935), found larger effects if the injections were continued for five days but this adds greatly to the length of the experiment.

Ideally, in every assay a control group of animals receiving saline, should be used. This was done frequently in the early experiments but subsequently, was not found to be necessary. Originally too, the standard preparation was used in every assay. It was, however, found that the conditions could be left so constant that the effects of the standard preparation showed little variation (Fig.3(a), and in later experiments the standard/

standard was used only once per fortnight. A similar diagram (Fig.3(b), shows that effects of the same doses of standard C.G. on the seminal vesicles, results in this case being much more variable than with the prostate. For instance, as shown in Fig.3(b) 2 I.U. had more effect on the seminal vesicles at the end of April than 4 I.U. had had earlier in the month; the sensitivity of the colony had, in fact, more than doubled in a fortnight. If the standard preparation were not used in each assay involving the seminal vesicles uncontrollable errors over a twofold range would occur. Fig.3(a) shows that much smaller changes occurred in the sensitivity of the prostates.

Methods of Extraction.

As mentioned above, untreated urine is used for the assay provided that the experiment is done within a reasonable time of collection of the specimen and that immediate and adequate refrigeration of the urine is undertaken. Oestrogens normally present in human urine do not interfere with the results and therefore their removal by appropriate methods, so important if one is using uterine weight or/

vaginal cornification for the assay, is not necessary. The evidence for this depends partly on experiments with extracts of urine which must be considered first. The yields obtained by various methods of extraction have been compared (Table 1), the urines having been collected from patients at different stages of pregnancy. Untreated urine and the extract of the same specimen of urine were always compared. The methods studied were:-

(i) Zondek's alcohol precipitation:- A portion of the 24-hour collection (usually 15c.c.) was acidified with glacial acetic acid to pH4 and distributed equally among three centrifuge tubes. To each tube 5 volumes of absolute alcohol were added and the solution allowed to stand overnight in the refrigerator. On the following morning the tubes were centrifuged, the supernatant fluid being discarded. The precipitate was washed with acetone, centrifuged and the supernatant fluid discarded, and was subjected to a similar process with ether. It was then dried thoroughly by compressed air and dissolved in 15c.c. of saline after which it was ready for injection.

In order to test the recovery with different/

different concentrations of the extract using this method the quantity of 0.9% saline in which the final precipitate was dissolved was varied, e.g., to concentrate by ten the final precipitate was taken up in 1.5c.c. of saline, thoroughly mixed by a glass rod, centrifuged when the precipitate was discarded and the supernatant fluid kept. The latter was subsequently diluted with 0.9% saline for comparison with an equivalent dose of native urine.

(ii) Acetone precipitation:- Acetone is more readily available than alcohol and has been found to give satisfactory results when used in the following way:-

Preliminary acidification of the urine to pH4 as above, is followed by precipitation by 5 volumes of acetone. The supernatant fluid is discarded and the precipitate washed with ether, centrifuged and taken up in a corresponding quantity of saline. As shown in Table 1, it is possible to concentrate ten times by this method without any loss of C.G.

(iii) Kaolin adsorption:- The technique used was that/

that of Scott (1941). Into 100c.c. graduated cylinder were put 25c.c. of urine and 25c.c. distilled water and 0.25c.c. bromphenol blue was added as indicator. The solution was adjusted to pH4 by the addition of 20% HCl. adding only 0.25c.c. of the acid at a time and shaking up the solution frequently until the colour changed from deep blue to a point where the blue almost disappeared - a faint bluish green colouration superimposed on the yellow background of the urine itself. 5c.c. of a well-shaken suspension of kaolin was added and the urine mixed thoroughly by inversion. The kaolin was allowed to flocculate and settle to a layer below the 30c.c. mark, the supernatant fluid was removed by suction and the kaolin suspension transferred to centrifuge tubes and centrifuged at 2,000 r.p.m. for three minutes. The supernatant fluid was decanted and the kaolin precipitate was thoroughly ground up into 10c.c. of n/10 NaOH by use of a thick glass rod. The emulsion became homogeneous. The solution was then centrifuged for ten minutes at 3,000 r.p.m. after which the supernatant fluids, 10c.c. in total volume, were combined and made slightly/

slightly acid to litmus by the addition of 10% HCl. drop by drop. Concentrated extracts were prepared by this method by the use of much larger initial volumes of urine, e.g., 100 or 200c.c. In such cases, 10c.c. of the kaolin suspension was used and the precipitate taken up in 10 or 15c.c. n/10 NaOH. Otherwise the procedure was exactly as outlined above.

(iv) Kaolin-Acetone-Ether Method:- For the majority of assays on pregnancy urine using the rat as the experimental animal, the tests are so sensitive that concentration of the urinary extracts is not required. In experiments on the gonadotrophins in the urine from non-pregnant subjects (F.S.H.) concentration of the urine is, however, necessary and a method has been developed in collaboration with Dekanski using first kaolin and then acetone. This method gives one-hundredfold concentration of the urine without significant loss of C.G. (Table 1).

The procedure is as follows:-

The pH of the specimen is adjusted to four using 20% HCl. The 24-hour collection of urine in a Winchester bottle, is made up to two litres by the addition of distilled water. 20g. of acid Kaolin/

Kaolin suspended in 100c.c. of distilled water is added. The mixture is put on the shaking machine for one hour to allow adsorption to occur and is then left overnight in the refrigerator.

On the following morning, the supernatant fluid is carefully removed using a suction pump and the kaolin precipitate distributed among four centrifuge tubes and centrifuged for ten minutes at 3,000 r.p.m. The supernatant fluid is then poured off.

Elution is effected with n/10 NaOH (freshly prepared each day); 50c.c. is used per litre of urine. This is divided among the centrifuge tubes and the precipitate is thoroughly ground and dissolved. The tubes are centrifuged for fifteen minutes at 3,000 r.p.m. The supernatant fluids, usually highly pigmented, are combined in a beaker and the pH is adjusted to six with 20% HCl. The volume of the solution is measured and 5 volumes of acetone added. An immediate precipitate forms. The solution is allowed to stand for two hours. Approximately three-quarters of the supernatant fluid is sucked off and the remainder of the fluid and/

and the precipitate are transferred to centrifuge tubes and centrifuged for five minutes at 3,000 r.p.m. The supernatant is poured off and the precipitate is washed with ether and centrifuged for five minutes at 3,000 r.p.m. Finally, the ether is poured off and the precipitate dried thoroughly by compressed air. The precipitate usually dissolves completely in 50c.c. of 0.9% saline. In some cases, smaller volumes of saline were used and an undissolved residue removed in the centrifuge. However, this led to loss of activity and suspensions were therefore injected when necessary.

The recovery of F.S.H. like the recovery of C.G. using this method, was very satisfactory. With non-pregnant urine the F.S.H. recovery was found to be better than that of the tannic acid method (Levin and Tyndale, 1937), and as good as, or slightly better than, Zondek's original alcohol precipitation method.(Loraine, 1949).

(v) Permutit method:- This was first described by Milton, (1946), after the observation of Katzman, Godfrid, Cain and Doisy (1943), that the gonadotrophins may be separated in biological fluids by adsorption/

adsorption on permutit and elution with weak alkali. Milton applied it to the Xenopus test.

A chromatographic column was prepared from a piece of glass tubing of bore approximately one inch diameter and length approximately fifteen inches, with a constriction at the bottom. A glass wool plug was put in the constriction. The tube was placed in a rubber bung which fitted into a Buchner filtration flask. Permutit "Decalco" brand, was poured into the top of the tube until a column of material, ten inches deep, was produced. The column was washed by pouring on dilute ammonia, dilute hydrochloric acid, and finally, water. The column was dried by applying suction and was then ready for use. The whole of the morning specimen of urine, e.g., 300c.c. was filtered and adjusted to pH 3.5 with dilute acetic acid using bromphenol blue as indicator. It was then transferred to a separating funnel and clamped above the column and the top of the funnel was opened to allow the urine to flow on to the column at a slow rate so that the 300c.c./

300c.c. passed through in about thirty minutes. Gentle suction was applied, if necessary. The column was washed with water until the washings were colourless, and finally sucked dry. Elution of the hormone was carried out by pouring on 10c.c. of 5% ammonia on to the column and allowing it to percolate through before suction was applied, and this was followed by a second elution with 5c.c. of dilute ammonia. The eluates were combined and aspirated to remove ammonia and the solution was then ready for injection.

In one experiment, this method was not found to give a good quantitative recovery and was subsequently abandoned.

TABLE 1/

TABLE 1

Comparison of yields of G.G.

using different extraction methods

Week of Pregnancy	Urine preparation injected.	Conc.	No.of rats per group.	Total dose/ rat in c.c.	Prostatic weight mg/100g. rat.
20th	Untreated	-	10	0.5	124
	Alcohol extract.	-	10	0.5	127
18th	Untreated	-	8	0.5	133
	Alcohol extract.	-	8	0.5	132
16th	Untreated	-	10	0.5	169
	Alcohol extract.	x10	10	=0.5	168
18th	Untreated	-	10	0.5	150
	Acetone extract.	-	10	0.5	144
20th	Untreated	-	10	0.5	174
	Acetone extract.	x5	10	=0.5	179
24th	Untreated	-	9	0.5	141
	Acetone extract.	x10	9	=0.5	139
32nd	Untreated	-	12	0.5	138
	Kaolin extract.	x2.5	12	=0.5	136
34th	Untreated	-	10	0.5	139
	Kaolin extract.	x10	10	=0.5	140
31st	Untreated	-	9	0.5	164
	Kaolin-Acetone-Ether extract.	x12.5	9	=0.5	162
36th	Untreated	-	12	0.6	177
	Kaolin-Acetone-Ether extract.	x100	12	=0.6	180
30th	Untreated	-	6	0.5	157
	Permutit Extract.	x20	6	=0.5	103

The effect of oestrogens in the test.

Evidence is presented below that the oestrogens in pregnancy urine do not interfere with the test on the male organs, but they would undoubtedly interfere with other tests for C.G. such as those depending on vaginal smears or uterine weight. In order to determine the usefulness of these methods of extraction in such tests, extracts were injected in oöphorectomised rats and observations made on vaginal smears. The rats were injected twice per day for two days and once on the third day, giving a total of five injections. In one such experiment, a sample of urine collected at the thirtieth week of pregnancy was estimated chemically to contain 16mg. of oestrogen per litre. A total dose of 1/80c.c. of this urine was given to each rat in five injections and smears were taken at 72, 84, 96, 108 and 120 hours after the first injection. Only a full squamous response was regarded as a positive reading. Two out of five rats gave positive smears. When oestrogens had been extracted by alcohol, or acetone, a total dose equivalent to 5c.c. of urine had no effect/

effect so that less than 1/400 of the original amount of oestrogen remained. An extract prepared by the kaolin-acetone-ether method as described above, could be injected in larger amounts due to ease of concentration and it was found that a total dose corresponding to 50c.c. of the same urine specimen had no effect on the smears so that in this case, less than 1/4,000 of the oestrogen remained. No oestrogen was in fact, detected in any sample treated with alcohol or acetone. The use of kaolin on the other hand, was not found to separate the oestrogens from C.G.

During the latter part of pregnancy, the urine contains large amounts of oestrogens and it is theoretically possible that these might interfere with the test since large doses of natural oestrogens are known to cause a fall in weight of the prostate, (Turner, 1948), and apparently paradoxically, stilboestrol may cause enlargement of the male organs under certain circumstances. (Fiske and Werthessen, 1948). The results shown in Table 1 provide strong evidence that the oestrogens in pregnancy urine do not, in fact, have either effect. These/

These urines were taken from women at various times during the last six months of pregnancy when the oestrogens are particularly liable to be present in high concentrations. Nevertheless, it was found that untreated urine had practically the same effect on the prostate as extracts from which the oestrogens had been completely removed. In order further to test this point, a special experiment was undertaken in which ten rats each received 3 I.U. of standard C.G. in three injections over three days and ten littermates received the same dose of C.G. plus a total dose of 49.36µg. of sodium oestriol glucuronide. This was calculated to be equivalent to what would be present in the urine in the later stages of normal pregnancy. The results are shown in Table 2. It will be seen that the average weights of the prostate in the first group was 155mg. per 100g. and in the second, 154mg. per 100g. It is evident, therefore, that the amounts of oestrogen normally present in pregnancy urine are insufficient to affect the weight of the prostate.

TABLE 2./

TABLE 2

Effect of oestrogen

on prostatic weight in immature rats 21 days old.

Group	Dose	Prostatic weight mg. per 100g.										Arith. mean.
I	3 I.U.	174	134	148	139	158	148	169	140	157	182	155
II	3 I.U. + 49.36γ oestriol	146	155	155	182	156	141	178	152	147	130	154

Solution injected contained 488μgm. oestriol in 10c.c.

A full comparison of the various methods used is shown in Table 3.

TABLE 3./

TABLE 3

Extraction methods for C.G.

Method	Quantitative recovery using prostatic weight calculated from Table 1.	Oestrogen removal.	Conc. used.	Time required	Comments
Alcohol Precipitation	100.4%	+	x5 x10	16hrs(if allowed to stand overnight)	Classical method of Zondek. Chief difficulty is shortage of supplies of alcohol.
Acetone Precipitation	99.5%	+	x5 x10	1½hrs (approx.)	Cheap and easy method giving as good a recovery as above. Ideal for routine use.
Kaolin Method	99.3%	-	x2.5 x10	2hrs (approx.)	Cheap method, more laborious than simple acetone precipitation.
Kaolin + Acetone	100%	+	x12.5 x100	16hrs (approx.)	Ideal method for concentrating urine and .·. very useful for F.S.H. estimations.
Permutit Method	40%	-	x20	8hrs (approx.)	Both the adsorption on permutit and the elution with NH_4OH seem to involve loss.

Fiske and Werthessen (1948), gave stilboestrol to immature rats in dosage of 0.5γ-100γ and found that it produced enlargement of the prostate and seminal vesicles in the animals. They concluded that the enlargement produced with a small dosage (0.5γ) was due to pituitary stimulation in the animal by the stilboestrol and the large dosage produced enlargement in virtue of the direct effect of the oestrogen on the fibro-musculature of the organs. They found that stilboestrol was more effective when given in divided doses than in one injection.

In view of the possible interference with the prostatic weight test by the therapeutic administration of stilboestrol to selected cases it was decided to repeat this experiment. Four groups, each containing eight 21-day old male rats were employed, each rat wherever possible, having littermates in the other groups. One group acted as controls and the other three groups were given respectively .9γ, 9γ and 90γ of stilboestrol in oily solution. As usual, the rats were injected subcutaneously once per day for three days, killed on the/

the fourth day and the organs were weighed on the fifth day. The results with the prostates and seminal vesicles are shown in Table 4 where it will be seen that stilboestrol in the dosage given did not, in the rats used, produce any enlargement of prostate or seminal vesicles.

TABLE 4/

TABLE 4

Effect of stilboestrol

on male organs in immature rats.

(a) Prostates

Group.	Dose of stilboestrol	Prostatic weight mg./100g.								Arith. mean.
I	Controls	99	86	88	115	79	109	93	86	94
II	0.9γ	85	94	111	100	103	85	105	87	96
III	9γ	83	80	108	93	99	96	88	83	91
IV	90γ	108	83	93	104	95	105	74	77	92

(b) Seminal vesicles

Group.	Dose of stilboestrol.	Seminal vesicles mg./100g.								Arith. mean.
I	Controls.	17.8	14.4	18.1	17.1	12.1	16.7	18.5	16.2	16.4
II	0.9γ	15.6	16.6	16.1	20.0	14.5	13.3	14.8	17.6	16.1
III	9γ	15.4	20.5	17.2	16.8	15.1	17.2	20.5	18.4	17.6
IV	90γ	16.0	18.0	20.0	21.8	18.4	20.0	13.0	15.4	17.8

C.G. IN NORMAL PREGNANCY

Clinical Studies.

Curves showing the changes in the amounts of C.G. in the blood and urine at different stages of normal pregnancy have been published by Cole and Saunders (1935), Smith and Smith (1936), Rowlands and Boycott (1938), Browne and Venning (1939), Siegler (1939), Rakoff (1939), Taylor and Scadron (1939), Jones et al.(1944) and Venning (1948). Of these workers only Jones et al.(1944) expressed their results in International Units. They used the rat uterus as the end-point of the assay and they estimated C.G. in the serum. Their individual readings varied over a tenfold range. There have been a few comparisons between the concentrations of C.G. in blood and urine (Evans, Kohl and Wonders, 1937; Siegler, 1939; Trettenero, 1936; Smith and Smith, 1935), but there appear to be no published estimates in International Units of the daily excretion of C.G. in the urine during normal pregnancy.

A total of 116 estimations of C.G. in the urine of normally pregnant women has been made. The prostatic weight method was employed throughout.

Four/

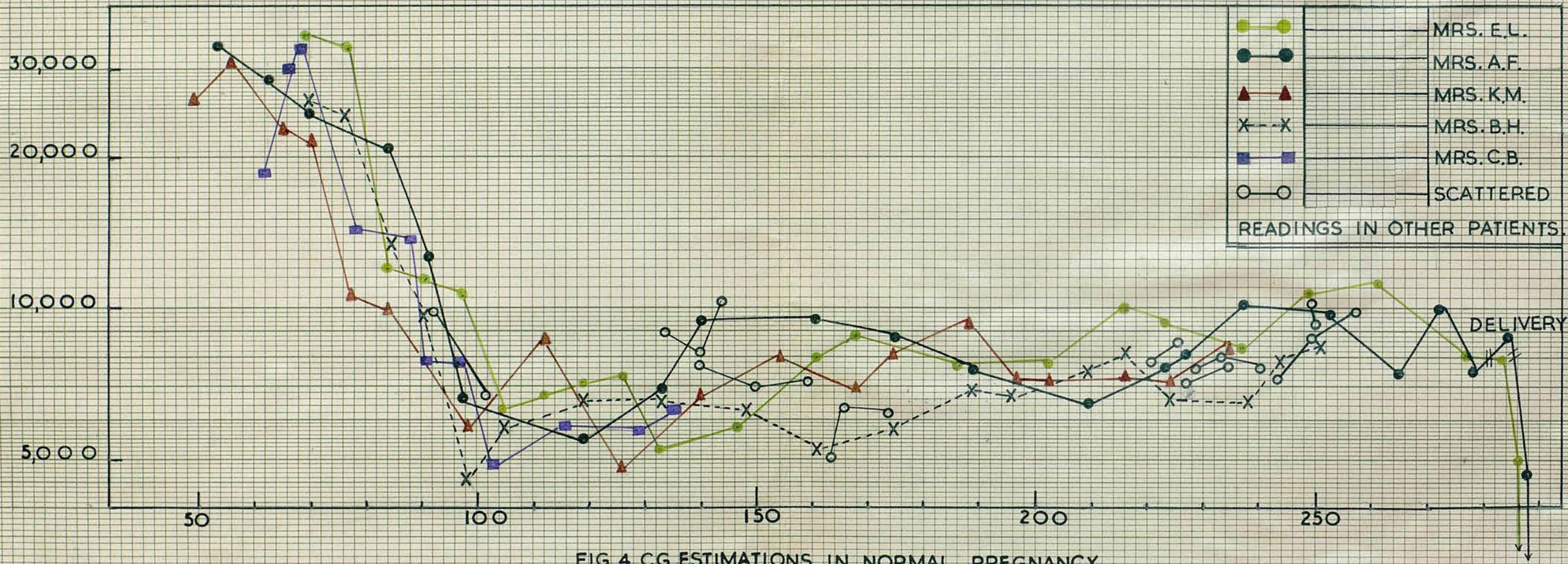

FIG. 4 C.G. ESTIMATIONS IN NORMAL PREGNANCY.

VERTICAL SCALE – C.G. IN I.U. PER 24 HOURS (LOG. SCALE)

HORIZONTAL SCALE – TIME IN DAYS FROM 1ST DAY OF LAST PERIOD.

Four patients were followed throughout the whole of pregnancy at approximately weekly intervals and scattered readings were obtained in other ten patients. The results are always expressed in I.U. of C.G. per 24-hour specimen of urine. This is thought to give a better estimate of the excretion of C.G. than would be obtained if the results were expressed merely as I.U. of C.G. per litre of urine. The estimations in the individual cases are shown in Fig.4 and Table 5. In Fig.5 are shown the mean results at fortnightly intervals during pregnancy along with the figure for the standard deviation of a single observation calculated for the period after the fourteenth week.

The estimations of the concentration of C.G. in the urine during normal pregnancy are quantitatively similar to those of Jones et al.(1944), of the concentrations in serum. Curves of the same general shape have been obtained by other workers who expressed their results in arbitrary "rat units", e.g., Venning and Browne (1936); Smith and Smith, (1936).

Very high readings, such as 20-40,000 I.U. per/

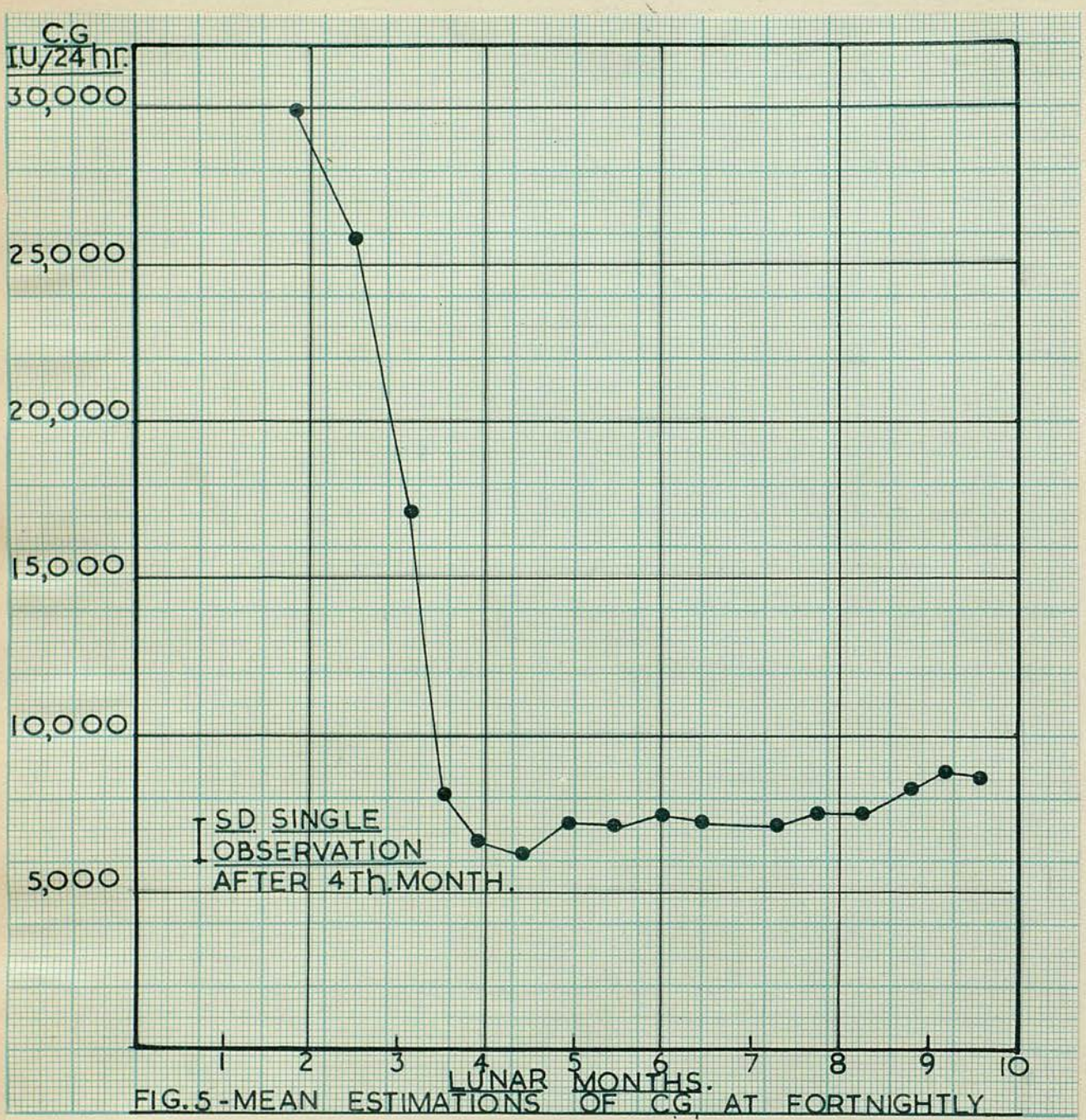

FIG. 5 - MEAN ESTIMATIONS OF CG AT FORTNIGHTLY INTERVALS DURING NORMAL PREGNANCY.

per 24-hours are obtained normally from the fortieth to the eightieth day of pregnancy as calculated from the date of the last menstrual period. This is followed by a gradual decline and by the hundredth day the "peak period" is past, the period of upsurge having been followed by a period of decline. From one hundred days onwards to the end of pregnancy the readings shown in Fig.4 remain at, or below, the 10,000 I.U. mark. In fact, of the 79 readings of C.G. made after the peak period, only one was above 10,000 I.U. and that a reading just before delivery of 10,980. The mean of these 79 estimations was $7,540 \pm 1,360$ (standard deviation of a single observation). From this, it may be calculated that the normal range (P=0.99) is about 4,000-11,000 I.U. per 24-hours. After one hundred days of gestation, it was found that all the readings from the normal women studied lay within this range. Estimations outside this range should therefore be regarded as pathological.

It will be seen (Fig.5) that the mean values fell to 6,270 I.U. per 24-hours at the seventeenth week and then gradually rose to 8,980 at the thirty-seventh week. In order to test whether this gradual/

gradual rise was significant, a comparison was made between the mean of the estimations taken from the fifteenth to twenty-second weeks of pregnancy and the mean of the estimations made from the thirty-second to the fortieth week. The figure for the latter group was found to be significantly higher than that for the former, ($P < 0.01$). It can be assumed, therefore, that with the approach of term the excretion of C.G. tends to rise slightly. In the cases followed after delivery, a rapid fall in the C.G. level occurred, the hormone dropping to below 1,000 I.U. per 24-hours by the third day of puerperium.

The figures quoted are taken from a relatively small number of patients, but they do appear to indicate that the normal variations in the amount of C.G. excreted per 24-hours during the later stages of pregnancy, are smaller than has generally been supposed, and that much of the apparent variation observed by previous workers has been due to the use of inaccurate assay methods. In addition to the relative accuracy of the method, depending on the prostatic weight, it has been shown that preliminary extraction/

extraction of the urine is not necessary as the oestrogens present in pregnancy urine do not interfere with the results. When oestrogen removal is an essential preliminary as it is when using, for example, uterine weights or vaginal smears for the assay, extraction of the urine should be carried out by acetone precipitation. This gives just as satisfactory a quantitative recovery as does alcohol, and acetone is much more readily available nowadays. The kaolin method, (Scott,1941) is quantitative but does not remove oestrogens. To do this, subsequent precipitation by acetone is necessary.

The actual function of the chorionic gonadotrophin which floods the tissues during pregnancy is still a matter of conjecture although no other hormone has been more extensively studied. Browne and Venning (1939), suggested that in women C.G. is essential for the maintenance of the corpus luteum and for the continuation of pregnancy. They considered the "peak period" to be the time at which the placenta was "taking over" from the pituitary the production of gonadotrophin. However, after the placenta assumes the function of oestrogen and progesterone/

progesterone production the C.G. excretion falls. This period from one hundred days onwards in pregnancy might well be called the era of placental dominance when the placenta can be regarded as a self-contained autonomous organ capable of functioning without the assistance of other endocrine glands.

The dramatic experiments of Hisaw (1944), using monkeys, are noteworthy in this connection. This worker was able to prolong the menstrual cycle and the secretory life of the corpus luteum in monkeys by injections of C.G. He therefore states that the action of C.G. is essentially "luteotrophic" in character. This effect could not be produced by pituitary luteotrophin which is believed to be identical with prolactin. Hisaw also showed, by histological examination of the endometrium and study of the sexual skin in monkeys, that chorionic gonadotrophin, unlike pituitary luteotrophin, could cause the secretion of progesterone and oestrogen. This is borne out by the clinical observation of Venning and Browne (1938), who showed that the administration of C.G. in the luteal phase of the menstrual/

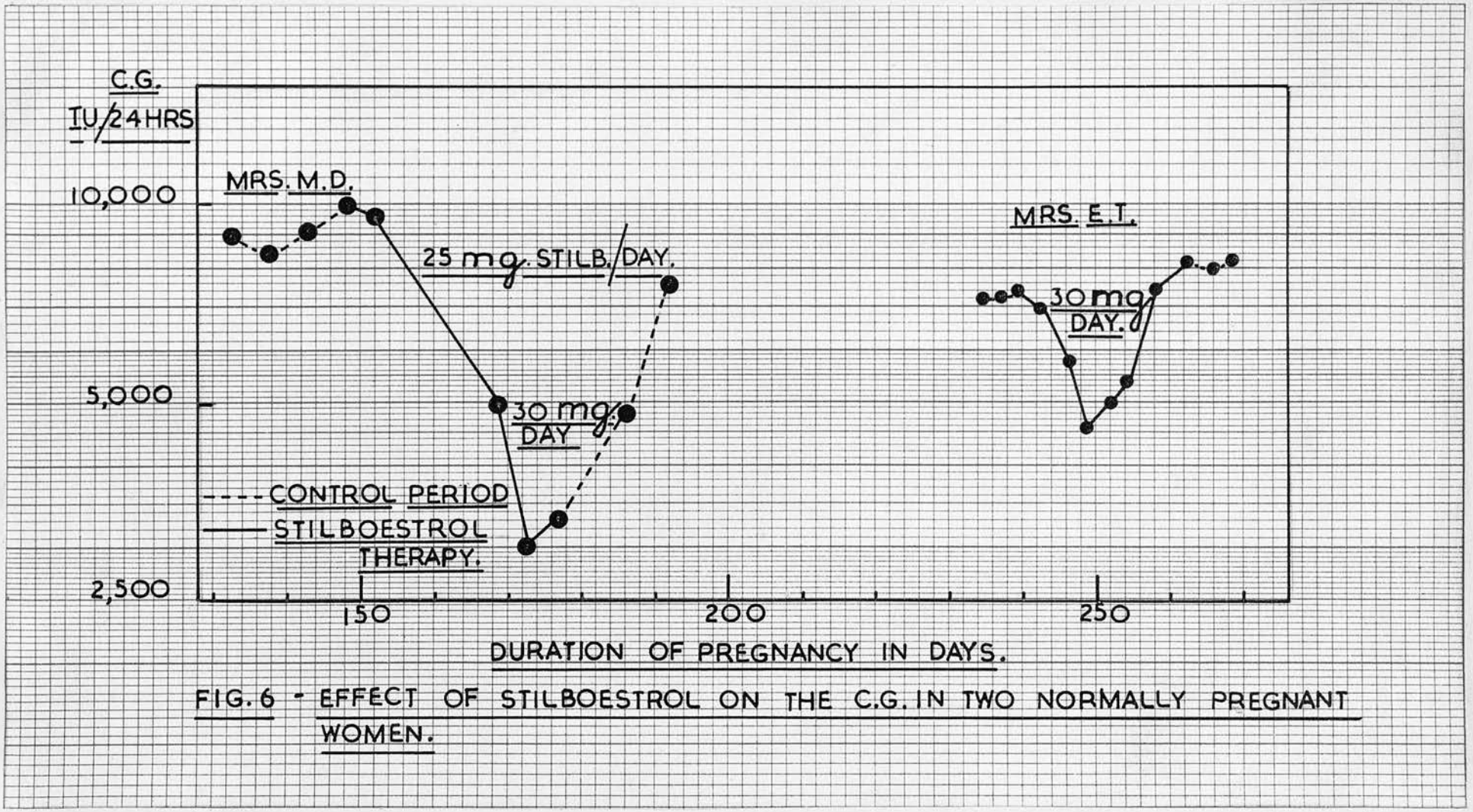

FIG. 6 - EFFECT OF STILBOESTROL ON THE C.G. IN TWO NORMALLY PREGNANT WOMEN.

menstrual cycle resulted in the excretion of an increased quantity of urinary pregnanediol.

Effect of stilboestrol in normal pregnancy.

The effect of stilboestrol on the C.G. readings is fully discussed in the section devoted to pregnancy in diabetes, stilboestrol having been administered to a proportion of the diabetic women considered. As essential preliminary, however, was to assess the effect of stilboestrol on the C.G. readings in normal women. The results of this experiment are shown in Fig.6. Stilboestrol was administered to the patient M.D.at the nineteenth week of pregnancy in dosage of 25mg. per day, increased after one week to 30mg. per day. A characteristic depression of the titre was noted, the level rising again on cessation of the treatment. The oestrogen was also given to the patient E.T. who was at the thirty-third week of pregnancy. The dosage of stilboestrol given was a constant one, viz. 30mg. per day. A modest initial depression was produced but on the same dosage the reading tended to climb back to a point a little above the original level./

level. This effect will be referred to in detail in the next section. From this study in two normally pregnant women it appeared that the sensitivity to stilboestrol was greater in early than in late pregnancy.

TABLE 5./

TABLE 5

C.G. READINGS in NORMAL PREGNANCY

(Method = Prostatic weight)

C.G. READINGS I.U. per 24-hours.

Patient	WEEK OF PREGNANCY. 7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
E.L.				35,050	32,740	11,934	11,452	10,782	6,312	6,781	7,075	7,196	5,116		5,689		7,970	8,780			6,680		7,696		9,800	9,200		8,165	
A.F.		33,860	27,540	24,816	24,240	21,117	12,562	6,568			5,432		6,780	9,350			9,468		8,552		7,665			6,355		7,796	8000	10,140	9,62
B.H.				25,340	24,660	13,485	9,660	4,490	5,820		6,510		6,470		6,280		5,184		5,620		6,800	6,776		7,225	8,090	6,485		8,341	7,87
K.M.	26,020	31,640	22,387	21,900	10,668	9,980		5,756		8,600		5,850		6,840		7,730		6,890	8,410		9,305		7,000	6,010		7,220	7000		
C.B.			18,320 30,830	31,310	14,626	13,730	7,770 7,850	4,908		5,956		5,570	6,200																
SCATTERED READINGS IN VARIOUS PATIENTS.							9,810	6,760						7,600	7,000	7,000										7,770	7800		
													8,900	8,000			4,800	6,200	5,758								7360 7840	7,420 7,410	
														8,000															7,20

24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	PUERPERIUM 2 days	PUERPERIUM 4 days
780			6,680		7,696		9,800	9,200		8,165		10,430		10,980	7,940	7,850	4,940	>1,000
	8,552		7,665			6,355		7,796	8,000	10,140	9,620		7,300	9,557	7,580	8,705	3,230	>1.000
	5,620		6,800	6,776		7,225	8,090	6,485		8,341	7,870							
890	8,410		9,305		7,000	6,010		7,220	7,000									
								7,770	7,800			9,000						
									7360	7,420								
200	5,758								7840	7,410								
											7,200	8,600	9,600					

PREGNANCY IN DIABETES

Introduction.

Before 1921, the occurrence of pregnancy in a diabetic woman was so rare that it was regarded as a medical curiosity. The great French physician, Bouchardat (1875), in his wide practice saw no cases of pregnancy in diabetics and observed "dans le nombre si considerable de diabétiques qui sont venus me consulter je n'ai pas memoire d'avoir vu une seule femme enciente." Similar was the experience of the great German clinician, Naunyn (1906), whose views on the dietetic treatment of diabetes mellitus held sway from 1898 to 1941.

Very low fertility rates of 2-6% due to the amenorrhoea resulting from uncontrolled diabetes in pre-insulin days were also reported by Lecorché (1885), and von Noorden (1909). Those few diabetic women who did actually conceive often did not survive the period of gestation, death occurring from complications, especially diabetic coma, often relatively early in pregnancy. The maternal mortality rate has been/

been estimated to be well above 30%, (Duncan, 1882; Williams, 1909), and the foetal loss rate has been put even higher, often approaching 50%, according to a review by Henley,(1947). In those days, therefore, pregnancy in a diabetic woman could well be described as a lethal complication to be dreaded both by patient and physician alike.

The discovery of insulin by Banting and Best,(1922), not only revolutionised the treatment of diabetes, but also increased fertility in diabetic women due to greatly improved control of the disease. Adequate medical and obstetrical care of these patients gradually reduced the maternal mortality rate from about 30% to 1-3%, (Kramer,1938; Lawrence and Oakley, 1942), so that from the maternal point of view, the risk to life of a pregnancy in a diabetic, is now very small. In fact, in Joslin's clinic, between January, 1936 and March, 1946 the maternal mortality was 0.4% in 271 cases, and in smaller series of cases reported by Shir (1939), and by Mengert and Laughlin (1939), and others, there was no maternal death rate at all.

The diabetic woman of today who requires investigation/

investigation for sterility is the exception rather than the rule, but, as pointed out by Barns,(1941), pregnancy in a diabetic woman is still relatively rare, due to some extent, to the fact that diabetes mellitus is much more frequent in women during the latter portion of the child-bearing period when pregnancy is less common.

Unfortunately, the foetal mortality in diabetic pregnancies unlike the maternal, has not shown any marked decline since the introduction of insulin and foetal wastage remains the great problem in obstetrical diabetes. In the pre-insulin days, it was 41%. Since the introduction of insulin, it has remained at approximately the same figure but much disparity of opinions exists among clinicians as to the exact definition of the term "foetal loss rate." For instance, Mengert and Laughlin (1939), considering only foetal deaths occurring in late pregnancy and deaths of babies in the neonatal period, had a foetal loss rate of only 18.2%, and using similar criteria, Miller, Hurwitz and Kuder, (1944), reported a foetal loss rate of 23.6%. Other workers, however, who included the whole course of pregnancy/

pregnancy and the neonatal period in their study, had substantially higher rates, e.g., Ronsheim (1933) had a foetal mortality rate of 64%, Herrick and Tillman (1938), of 43%, Barns (1941), of 44% and Lawrence and Oakley (1942), of 37%. In a recent paper, Gilbert and Dunlop, (1949), including in the term "foetal loss rate", all abortions, miscarriages, intra-uterine deaths, stillbirths and deaths occurring during the first fourteen days of the neonatal period, reported a foetal loss rate during the past five years of 51.4% in a total of seventy diabetic pregnancies. Barns and Morgan, (1949), using similar criteria reported an overall foetal mortality of 55% in their series of fifty-eight diabetic pregnancies.

According to White (1946), abnormalities occurring in diabetic pregnancies can be divided into five main groups - maternal, obstetrical, chemical, foetal and placental.

The maternal abnormalities include vascular disease, a frequent concomitant of diabetes and the complication par excellence of diabetes in the older age groups, and hypo-ovarianism as indicated by menstrual/

menstrual irregularities and oligo or amenorrhoea.

On the obstetrical side, sometimes uterine irritability and sometimes inertia occurs. Contraction rings are relatively frequent. Abortions and miscarriages are all too common and Joslin (1946), places the incidence of pre-eclampsia as high as 40% in his series of cases.

From the chemical point of view, the diabetic woman has a low renal threshold for glucose and often a profound disturbance of water balance as indicated by a tendency to oedema and hydramnios. The phenomenon described by White (1943), under the term "sex hormone imbalance" forms an important part of this discussion and will be considered in some detail below.

The most important foetal abnormality is the frequent finding of very large babies. These infants can be described as "long, wet and heavy" often weighing up to 10lbs. at birth and tending to lose weight rapidly after delivery due to evacuation of oedema. In addition, these babies often show splanchnomegaly, (White and Hunt, 1943; Miller and Wilson, 1943), affecting especially the liver, spleen and heart. Congenital abnormalities, including/

including dwarfism, syndactylism, congenital dislocation of the hip, club foot and spina bifida are also relatively frequent. Many paediatricians have emphasized the tendency of these infants to develop hypoglycaemia (Wiener, 1924; Lawrence, 1929; Platou, 1942; Woodrow, 1942; Bigby and Jones, 1945), but White (1946), considers that the condition should be regarded as an instability of the blood sugar rather than actual hypoglycaemia.

The placenta of the diabetic pregnancy is usually larger than normal with a thickened cord and often numerous infarcts.

Set in this background, therefore, of high foetal loss rate in diabetic women the results of White (1943; 1946), using hormonal therapy in these cases are very striking indeed. The endocrine assays undertaken in these women were estimation of the serum chorionic gonadotrophin in 21-day old rats using corpora lutea as the end-point of the assay, urinary pregnanediol by the Venning and Browne (1937), and Astwood (1941) methods, and in a few cases, serum oestrogens by biological methods. By this means, it was found that diabetic women could be divided into two brood groups, each group carrying/

carrying an entirely different prognosis. In 64 cases in whom the hormonal readings were classified as normal, the foetal survival rate was 97% and the incidence of premature deliveries was 0% and of pre-eclampsia 2%. 54 cases were classified as having an abnormal hormonal balance as indicated by a high serum C.G. a low serum oestrogen and a low urinary pregnanediol. In these cases, the foetal survival rate was only 52%; pre-eclampsia occurred in 50% and premature delivery in 40%, many of the patients delivering very large babies.

Of the estimations done, that of the C.G. appeared to be the most important. It appeared therefore, that the diabetic women who went normally to term and delivered a normal baby had normal C.G. readings throughout. Those with abnormally high C.G. readings had a much greater tendency to some accident during pregnancy and usually delivered a very large baby. A rise in C.G. level, therefore, could be regarded as the harbinger of an impending disaster. It is interesting to note that such cases are exactly analogous to a proportion of pre-eclamptic women reported by Smith and Smith, (1948), who/

who said that the rise in C.G. in such cases is a "compensatory" phenomenon due to lack of oestrogen and progesterone.

If, in these diabetic women, the C.G. level could be brought down to normal limits by oestrogen therapy, sometimes in association with progesterone where the pregnanediol was low, the prognosis in this group was greatly improved. In fact, in 157 cases classified as having "hormonal imbalance" during pregnancy and treated with oestrogens and progesterone, the foetal survival rate rose to 90%, premature deliveries fell to 15% and pre-eclampsia occurred only in 5%. In addition, the majority of patients in this group delivered normal sized babies. Although the estimation of "foetal loss rate" in the series of cases studied by White did not include abortions before the twenty-fourth week of pregnancy, the results were so striking that further investigation of the problem seemed necessary.

The series of diabetic pregnancies so far studied in Edinburgh, is as yet, too small adequately to assess the effect of stilboestrol in reducing the foetal loss rate and the present study is mainly concerned/

concerned with the C.G. readings in diabetic women and the effect of stilboestrol on those readings. In all, 15 diabetic patients have been considered, 9 of whom have had C.G. estimations at approximately weekly intervals from the time of diagnosis of pregnancy. In the other 6, more scattered readings were obtained. Stilboestrol was given to those diabetics, who, after one hundred days of gestation, consistently showed readings above the normal level of 10,000 I.U. per 24-hours, the prostatic weight method being used for all estimations. Six cases received stilboestrol using this criterion, but one case (No.XIII), was given the drug on an empirical basis.

A detailed review of the clinical and obstetric findings in these cases is presented below in association with the C.G. readings in each patient. The subject is elaborated further in the discussion, where an attempt is made to correlate clinical and endocrinological aspects of the problem.

Clinical Studies.

Case I - Mrs. O.B. Age 27.

Previous obstetric history:- Para 1 in 1946: male child weighing 6lbs. 12oz. Delivery was normal and the patient went on working until the day before delivery. She had one hypoglycaemic episode during this pregnancy.

In the present pregnancy the patient was first seen at the sixteenth week. The duration of diabetes mellitus was seven years. In the family history it was noted that one maternal aunt had died of diabetes.

As regards the complications of diabetes the patient had a severe hypoglycaemic coma at the thirty-sixth week from which she recovered spontaneously. There was mild ketosis during the twenty-fifth and twenty-sixth week.

During her pregnancy the patient had no hydramnios, no vomiting and no evidence of toxaemia. An obstetrical complication was present, the presentation being a breech which however, subsequently underwent spontaneous version.

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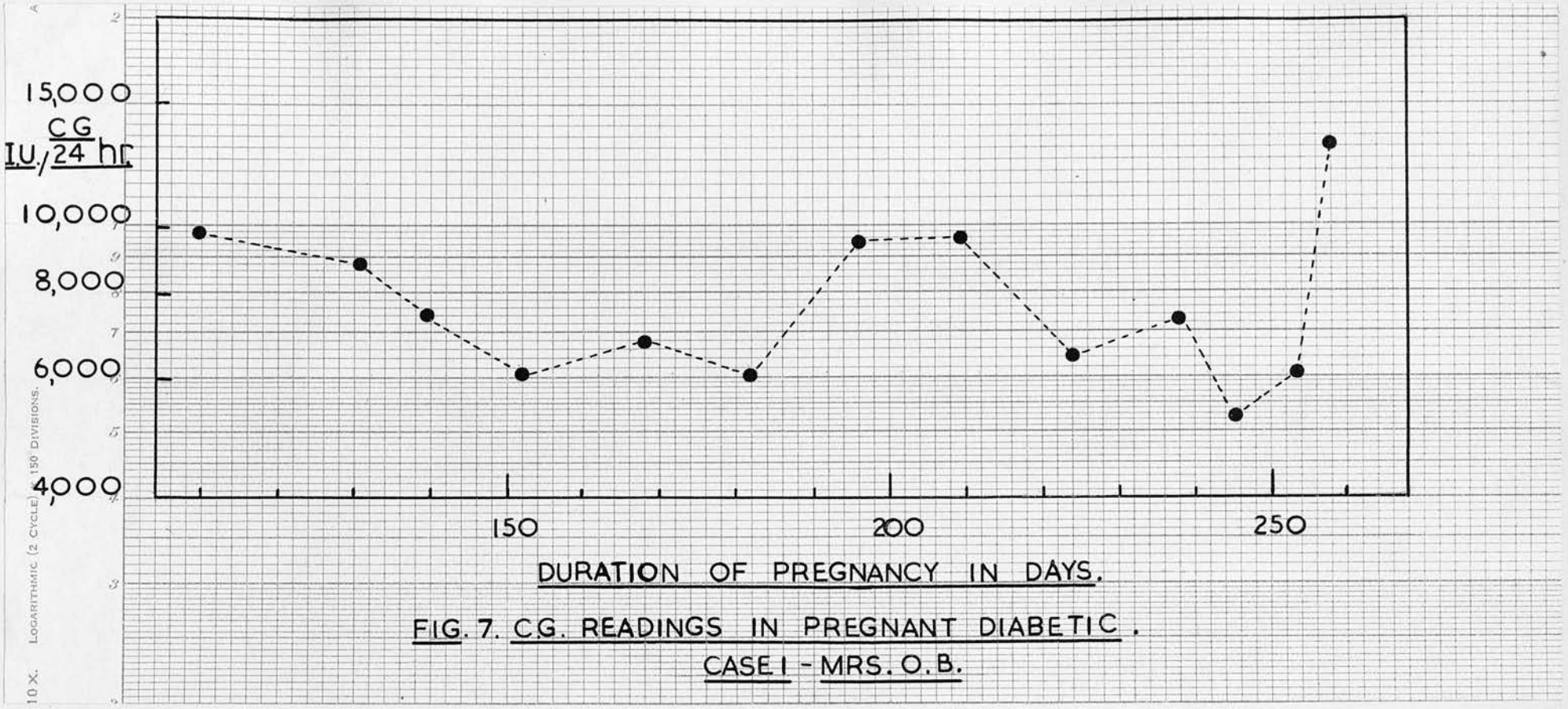

FIG. 7. C.G. READINGS IN PREGNANT DIABETIC.
CASE I - MRS. O.B.

On the biochemical side the blood sugar levels taken weekly at noon showed considerable fluctuations, the highest figure being 200mg. per cent. The renal threshold was 140-150mg. per cent. Insulin requirements (ZP + soluble) rose from 34 Units per day at the sixteenth week to 66 Units at the thirty-fifth week.

The diet during pregnancy consisted of 2,600 calories per day with 194g. of carbohydrate.

Delivery occurred on 17th February at the thirty-eighth week of pregnancy by the vaginal route following a medical induction. The labour was uneventful and lasted 4½ hours and the puerperium too, was uneventful the patient being discharged on the ninth day.

The baby was a male child weighing 6lb. 15½oz. The placental weight was 1lb. 8 oz. The infant throve well. Lactation and breast feeding were established.

C.G. Readings were entirely normal throughout the whole of pregnancy except for the last reading obtained. This figure was 13,000 I.U. per 24-hours at the thirty-seventh week. No hormonal therapy was given in this case. (Fig. 7 and Table 6).

TABLE 6/

TABLE 6

C.G. Readings in Pregnant Diabetic

Name of Patient - Mrs O.B.

Week of Pregnancy	C.G. I.U. per 24-hours.	Stilboestrol
16	9,644	None given
18	8,760	
19	7,378	
21	6,140	
23	6,690	
25	6,058	
27	9,380	
29	9,370	
31	6,490	
32	7,200	
33	7,200	
34	5,300	
35	6,000	
37	13,130	

Case II - Mrs. M.T. Age 20.

Previous obstetric history:- The patient was a primipara. During this pregnancy she was first seen at the tenth week. At this time diabetes had been present for 14½ years. In the family history it was found that diabetes was present in an elder brother of the patient and her husband's grandmother also suffered from the disease.

She showed no retinal, cardiovascular or neurological complications of diabetes. Mild hypoglycaemic attacks, occurring during pregnancy, were easily controlled and a transient ketosis occurred at the thirtieth week due to vomiting.

The pregnancy was not complicated by hydramnios or toxaemia but the patient had fairly severe vomiting in the first few weeks of pregnancy although this did not actually amount to the condition of hyperemesis gravidarum. Vomiting recurred at the thirtieth week of pregnancy for no apparent reason and this was associated with an evanescent ketosis.

The diet given during pregnancy almost amounted to a "free diet" with over 250g. of carbo-hydrate/

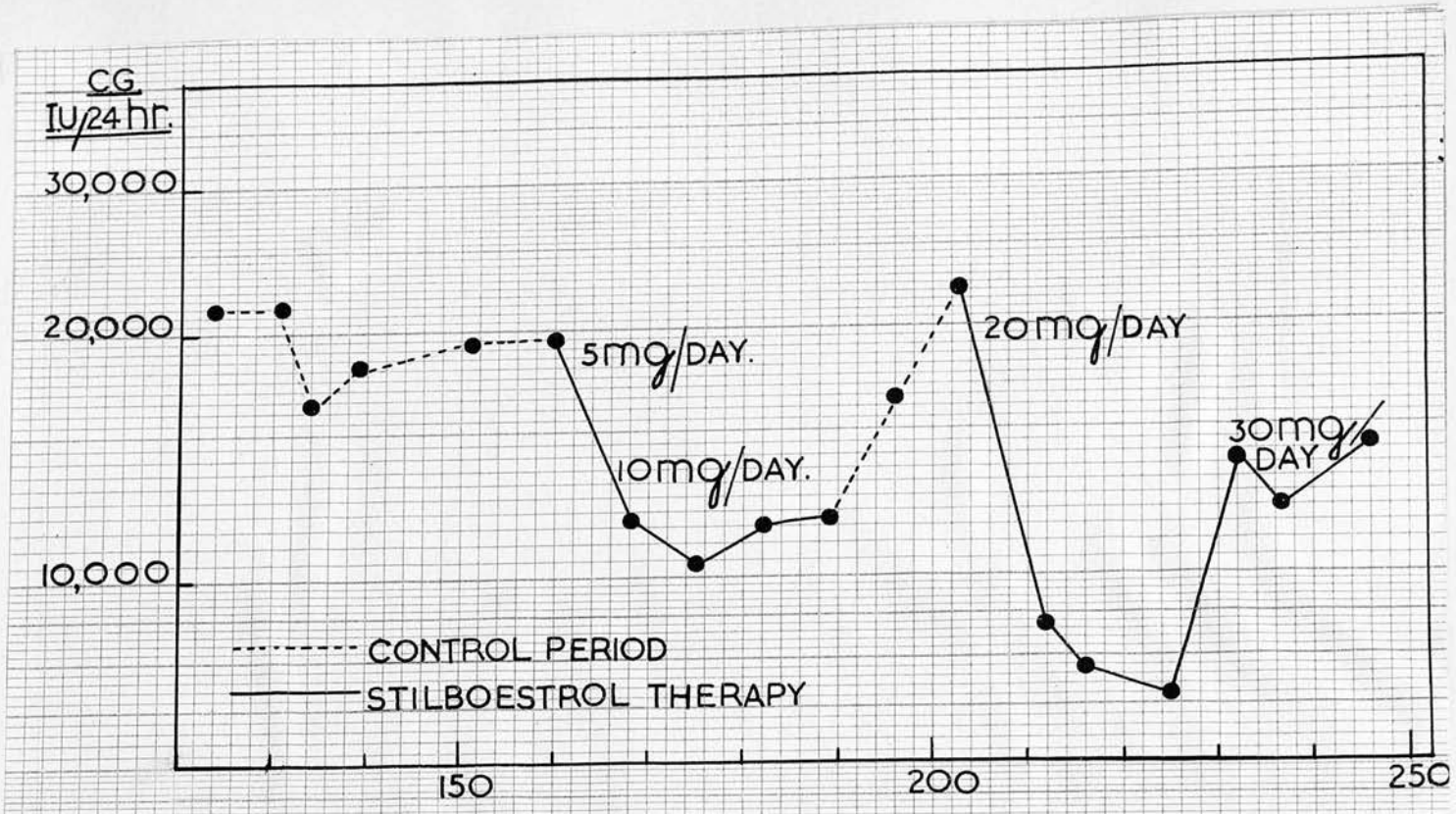

FIG.8. C.G. READINGS IN PREGNANT DIABETIC TREATED WITH STILBOESTROL.

CASE II - MRS. M.T.

carbohydrate per day. Blood sugars in the patient taken at noon were very variable, the highest figure recorded being 300mg. per cent. Total insulin requirements (Z.P. + soluble) rose steadily during pregnancy from 50 Units per day at the tenth week to 92 Units per day at the thirty-sixth week.

The patient was delivered on 8th January, 1949 at the thirty-sixth week by a classical Caesarean section, a spinal anaesthetic being employed. The operation and puerperium were uneventful.

The baby was a female, its birth weight being 6lb. 7 oz. Its blood sugar at birth was 134mg. per cent. Breast feeding was established and continued until the patient was discharged from hospital on the seventeenth day of the puerperium.

C.G. Readings: These are shown in Fig.8 and Table 7. It will be seen that estimations made from the eighteenth to twenty-fourth weeks of pregnancy showed abnormally high figures. Stilboestrol was accordingly administered orally to this patient in relatively small dosage commencing at the twenty-fifth week. A significant fall in the/

the excretion occurred with 5mg. per day, increased after one week to 10mg. per day, but on cessation of the treatment the reading rose to slightly above its original level. Subsequent therapy, with larger dosage of stilboestrol, was commenced at the thirtieth week of pregnancy and again a dramatic fall in C.G. level was observed. However, when the same dosage was continued over some weeks an "escape" phenomenon occurred, the reading tending to climb back to its original level just before delivery. Although the C.G. readings were high neither the placenta nor the baby were overweight considering the time of delivery.

TABLE 7./

TABLE 7

C.G. Readings in Pregnant Diabetic

Name of Patient - Mrs. M.T.

Week of Pregnancy	C.G. I.U. per 24 hours	Stilboestrol
18	21,240	
19	21,180) 18,470)	
20	18,120	
22	19,200	
23	19,200	5mg. per day +
24	11,920	10mg. per day
25	10,620	
26	11,600	Stop stilboestrol
27	11,870	
28	16,440	
29	22,430	
30	9,730	20mg. per day
31	7,856	"
32	7,230	"
33	13,900	30mg. per day
34	12,480	"
35	13,900	"

+ In this and subsequent tables, the figures refer to the oral administration of stilboestrol. Parenteral therapy when employed is indicated in the appropriate tables.

Case III - Mrs. H.E. Age 21

The patient had no previous children. In her pregnancy she was first seen at the twentieth week. Diabetes mellitus had been present for 2½ years and there was a strong family history of the disease, the patient's father having died of the condition and a paternal uncle having contracted it some years previously.

During her pregnancy she had no hypoglycaemic episodes and no ketosis. A moderate degree of hydramnios developed at the thirty-fourth week but there was no albuminuria, hypertension or oedema.

Blood sugar levels fluctuated considerably throughout pregnancy, the highest reading obtained being 242mg. per cent.

The diet consisted of 2,500 calories per day with 190g. of carbohydrate. The renal threshold was 140mg. per cent. Insulin requirements rose from 18 Units per day at the eighteenth week to 40 Units per day at the twenty-fourth week. They then remained constant until the thirty-third week and then surprisingly enough began to fall reaching a level of 17 Units per day at thirty-six weeks. This was the only case in whom a fall

in/

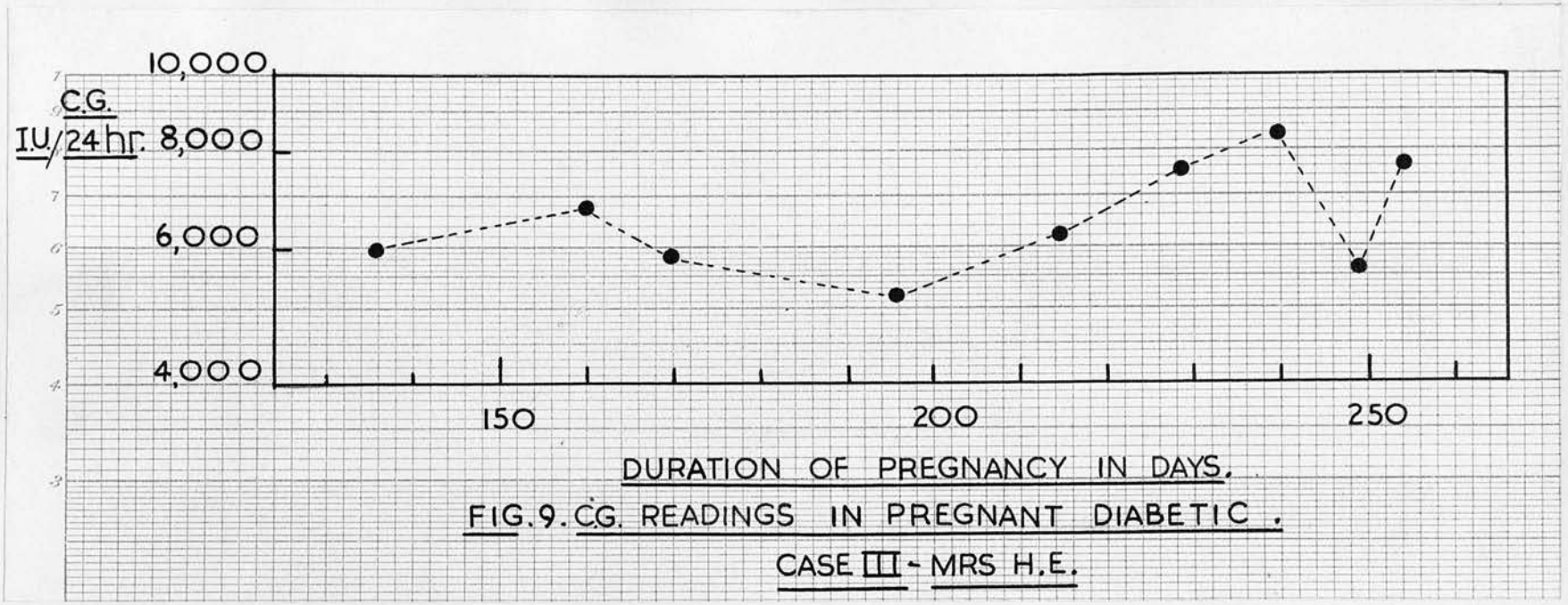

FIG. 9. C.G. READINGS IN PREGNANT DIABETIC.
CASE III - MRS H.E.

in insulin requirements occurred during pregnancy.

The patient was delivered on 12th February, 1949. at the thirty-eighth week of pregnancy. It was a vaginal delivery, low mid cavity forceps being employed. Labour lasted 3½ hours following a medical induction. The puerperium was uneventful, the insulin requirements gradually falling, till on discharge, only 10 Units of Z.P.1 per day were required.

The baby, a male child, weighed 7lb.1½ oz. at birth and the weight of the placenta was 1lb. 5 oz. The child was normal in appearance and its general condition was satisfactory. Its blood sugar at birth was 70mg. per cent. The baby went home in good condition on the ninth day of the puerperium.

C.G. Readings: (see Fig.9). These were entirely normal and very steady throughout the pregnancy. The highest reading obtained was one of 8,310 at the thirty-fourth week. No hormonal treatment was given. Results are shown in Table 8.

TABLE 8./

TABLE 8

C.G. Readings in Pregnant Diabetic

Name of Patient - Mrs. H.E.

Week of Pregnancy	C.G. I.U. per 24-hours	Stilboestrol
20	5,900	None given
22	6,680	
24	5,750	
26	5,085	
28	6,200	
32	7,680	
34	8,310	
35	5,582	
37	7,660	

Case IV - Mrs. C.B. Age 34.

Previous Obstetric History: para 2.

(a) Miscarriage at 2½ months in 1942.

(b) Live Birth in 1947: the birth weight of the baby was 7lb. 6 oz. and the child is alive and well.

In the present pregnancy this patient was first seen at the twenty-fifth week. Diabetes had been present for nine months. There was no family history of the disease. She had a few mild hypoglycaemic episodes during her pregnancy, but these were easily controlled. A transient ketosis developed at the thirtieth week due to vomiting after starting stilboestrol therapy. Numerous obstetrical complications occurred including a malpresentation, in this case, a transverse lie. In addition, she had a premature labour at the thirty-sixth week and a uterine constriction ring developed during labour. There was, however, no toxaemia and no hydramnios.

The maximum noon blood sugar recorded was 193mg. per cent. The diet during pregnancy was 2,500 calories with 200g. of carbohydrate and the renal threshold was approximately 100mg. per cent. Insulin/

Insulin requirements gradually rose during pregnancy from 27 Units per day at the twenty-fifth week to 40 Units at the thirty-sixth week.

Delivery was by the vaginal route at thirty-six weeks. The unusually painless labour was not recognised until the cervix was fully dilated. At this time, the foetus lay transversely. Lateral version was performed with delivery of the legs and breech. Unfortunately, however, a constriction ring developed before the head could be delivered and relaxation under chloroform was too late to save the child. The puerperium was normal, lactation being suppressed by dienestrol.

The baby was a male infant stillborn weighing 4lb. 11oz. On post-mortem examination the islets of Langerhans were much more extensive than usual.

Comment: It is unlikely that the loss of this child could be attributed to the diabetes alone as the death was due mainly to an unfortunate obstetrical complication.

C.G. Readings:/

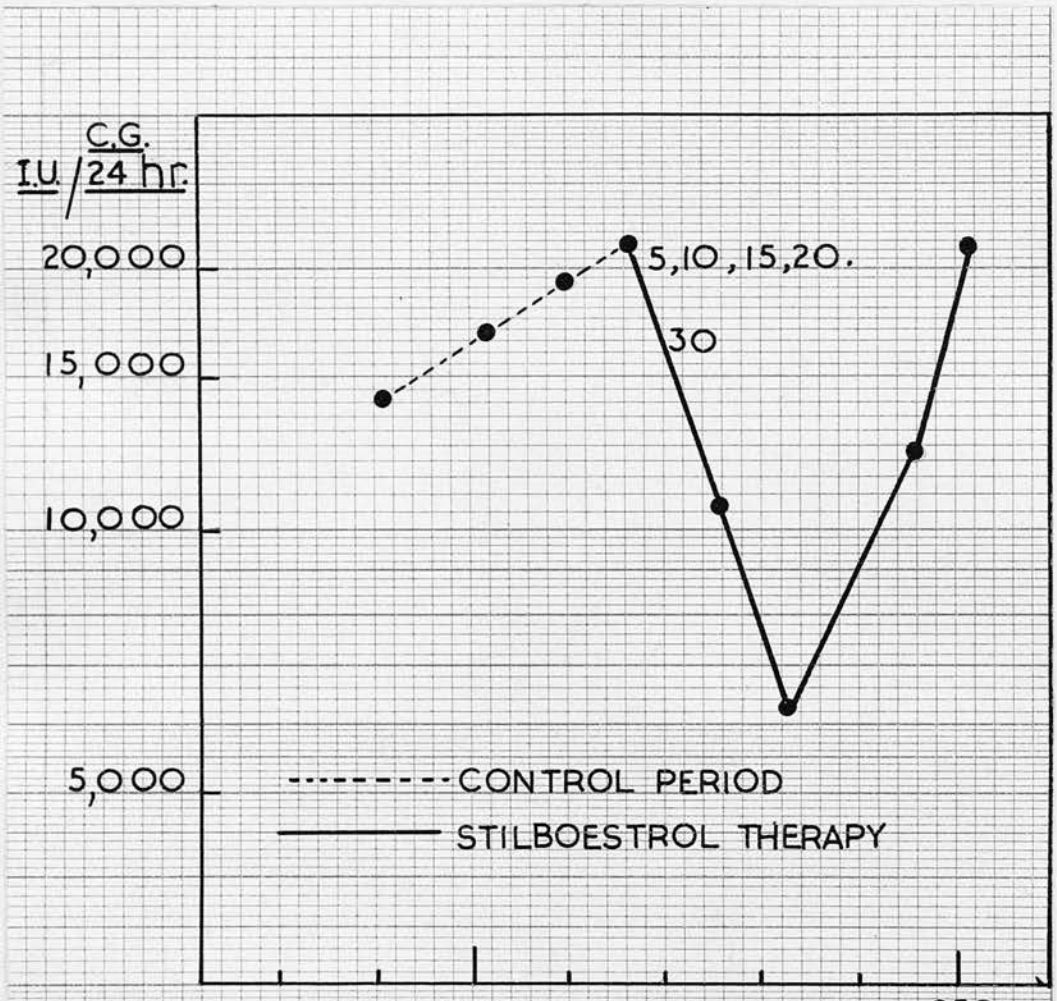

FIG. 10 . C.G. READINGS IN PREGNANT DIABETIC .

CASE IV - MRS. C.B.

IN THE PERIOD OF STILBOESTROL THERAPY

THE FIGURES INDICATE DOSAGE IN mg PER DAY

C.G. Readings: Abnormally high C.G. readings were found when this patient was first seen at the twenty-fifth week of pregnancy. Stilboestrol was commenced at the thirtieth week and the dosage was rapidly increased by 5mg. per day to 30mg. per day and maintained at this level. An initial fall in C.G. readings from approximately 20,000 I.U. per 24-hours to 6,500 I.U. was obtained, but the titre rose to its original level just before delivery, a constant dosage of the drug being maintained. (Fig.10 and Table 9).

TABLE 9/

TABLE 9

C.G. Readings in Pregnant Diabetic

Name of Patient - Mrs. C.B.

Week of Pregnancy	C.G. I.U. per 24-hours	Stilboestrol
27	14,160	
29	16,800	
30	19,240	5 10 15 20 mg. per day
32	20,850	30mg. per day
33	10,410	"
34	6,470	"
35	12,560	"
36	21,130	"

Case V - Mrs. M.Be. Age 42

Previous obstetric history: This patient was para 4.

(a) 1929 - Female, weighing 9½lbs. at birth
(b) 1931 - Male, " 12½ " " "
(c) 1935 - Female, " 10¼ " " "
(d) 1947 - Female, " 6 " " "

During the present pregnancy, the patient was first seen at the twentieth week. Diabetes had been present for five years. It was noted that the patient's father had had a mild form of diabetes for twenty-eight years.

During the pregnancy, there was no hypoglycaemia and no ketosis. Neither hydramnios nor toxaemia was present. The highest noon blood sugar reading was 133mg. per cent. The diet given was 2,600 calories with 200g. of carbohydrate. The renal threshold was 90-100mg. per cent. No insulin was required.

The patient was delivered on 2nd November, 1948 at forty-one weeks. An emergency Caesarean section/

section was undertaken as a previous medical induction had failed. The presentation was a transverse lie. A stormy puerperium ensued, an aspiration pneumonia developing. This was successfully treated by sulphonamides and penicillin. The weight of the baby at birth was 9lbs. It was a normal, healthy child which throve well.

Comment: The interesting feature in this middle-aged obese, relatively mild diabetic, was the fact that she had had two babies previously, both of which weighed over 10lbs. at birth. This occurred long before the development of clinical signs of diabetes and must therefore be regarded as taking place in the "prediabetic" phase of the disease.

C.G. Readings:- Estimations were made from the twentieth week onwards and were relatively normal until the twenty-fourth week when a sharp rise occurred. A transitory depression was obtained by stilboestrol in dosage of 20mg. per day originally, but again an "escape" phenomenon occurred, and even although the dosage was increased to 30mg. per day/

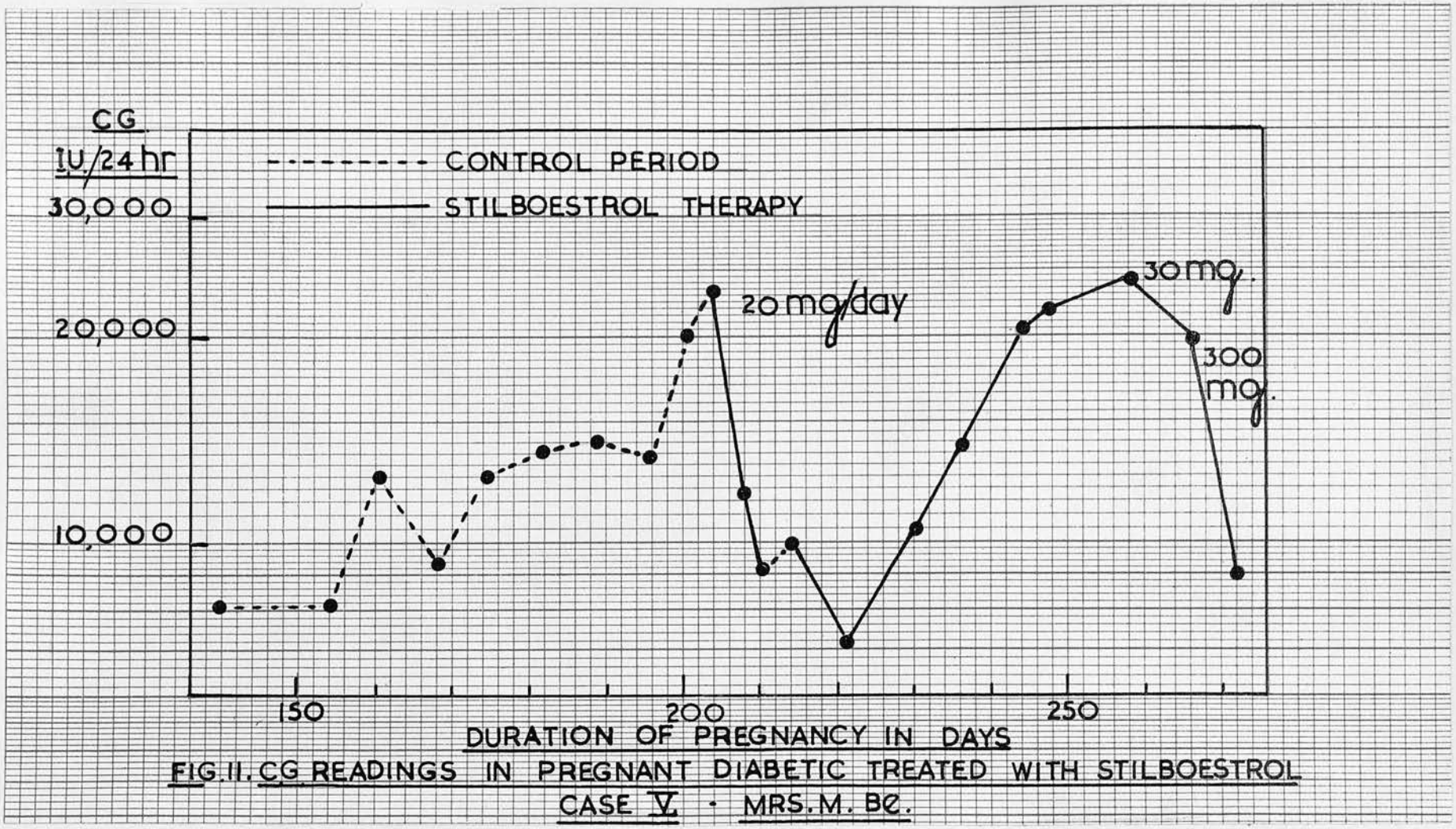

FIG. II. CG READINGS IN PREGNANT DIABETIC TREATED WITH STILBOESTROL
CASE V · MRS. M. Bc.

day at the thirty-fifth week, the level was little affected. A dose of 300mg. given to induce labour approximately halved the C.G. reading. (Fig. 11 and Table 10).

TABLE 10/

TABLE 10

C.G. Readings in Pregnant Diabetic

Name of Patient - Mrs. M.Be.

Week of Pregnancy	C.G. I.U. per 24-hours	Stilboestrol
20	8,109	
22	8,150	
23	12,710	
24	9,460	
25	12,800	
26	13,630	
27	13,960	
28	20,000) 23,000)	20mg. per day
29	11,820	"
30	9,160	"
31	10,264	"
32	7,260	"
33	10,600	"
34	13,680	"
35	20,650) 21,270)	30mg. per day
38	24,340	"
39	20,720	"
40	9,020	300mg.

Case VI - Mrs. M. Ba. Age 29.

The previous obstetric history was as follows:-

(a) 1946: stillbirth at 8 months following appendicectomy.

(b) 1947: Abortion at 4 months following diabetic coma.

This patient was first seen at the tenth week of pregnancy. Diabetes had been present for ten years. There was no family history of the disease.

During pregnancy slight hydramnios was present but no toxaemia developed.

The diet given was 2,300 calories with 200g. of carbohydrate, 105g. of protein and 121g. of fat. The highest noon blood sugar obtained was 300mg. per cent, the renal threshold being 130-160mg. per cent. Insulin requirements rose from 52 Units at ten weeks to 95 Units at thirty-four weeks.

The patient was delivered by Caesarean section at the thirty-seventh week using spinal anaesthesia. The placenta weighed 1lb. 2oz. The puerperium/

puerperium was uneventful but a rapid fall in insulin requirements occurred.

The baby at birth weighed 6lb. loz. It was normal in appearance showing no oedema. Feeding of the child was always difficult and oesophageal tube feeds were employed. Unfortunately, the infant inhaled a feed one week after birth and developed an aspiration pneumonia from which it died on 24th November, 1948.

Comment: The loss of this baby must be attributed to some extent to the diabetes, as the child was weakly and had difficulty in feeding. The death of the infant was all the more tragic in view of the previous obstetric history.

C.G. Readings:- This patient was followed very carefully from early pregnancy and in all forty-one estimations of C.G. were made. The abnormally high readings at the tenth week, i.e., 65,000 I.U. per 24-hours, were originally thought to indicate a delayed peak period, but the subsequent readings remained consistently high. Stilboestrol in dosage of/

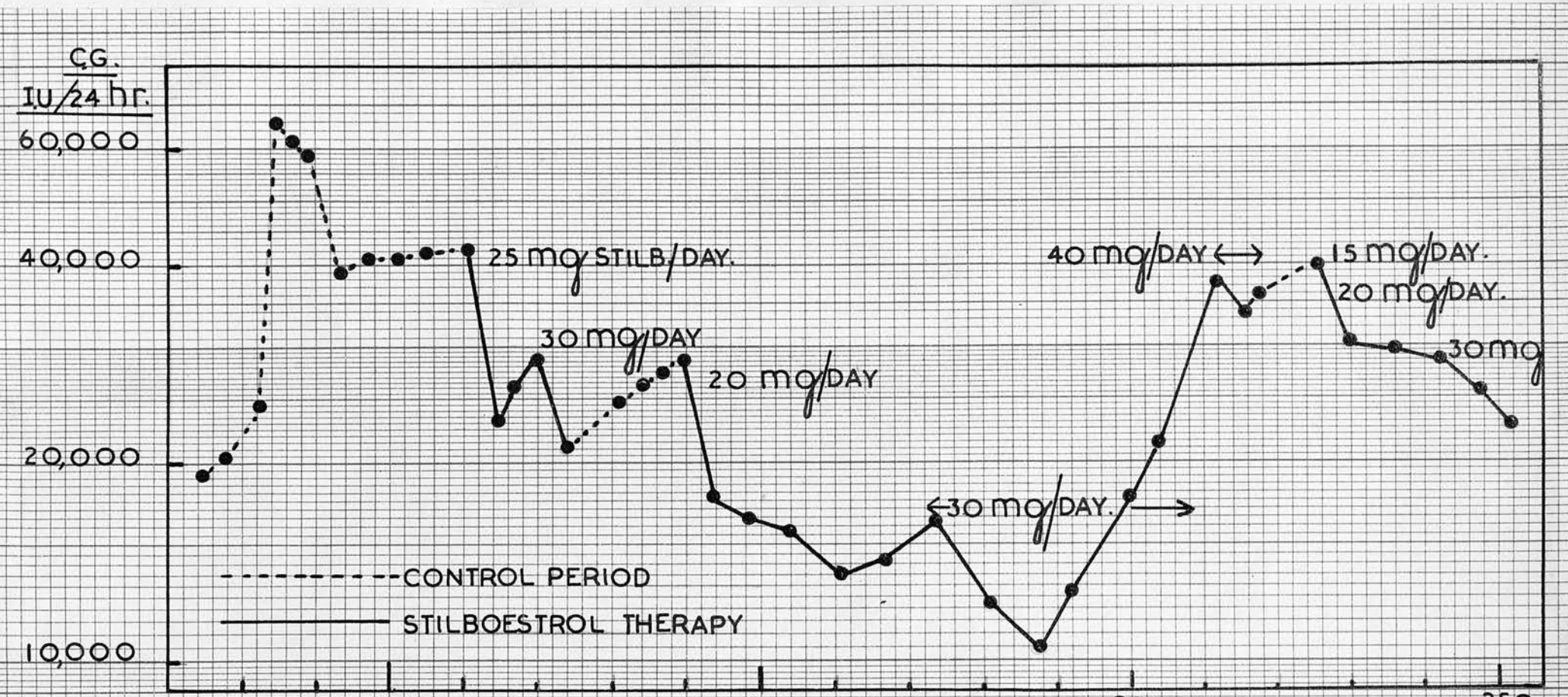

FIG. 12. C.G. READINGS IN PREGNANT DIABETIC TREATED WITH STILBOESTROL.
CASE VI - MRS. M. Ba.

of 25mg. per day followed by 30mg. per day six days later, was given for experimental purposes, starting at the sixteenth week. The drug was stopped at the end of the seventeenth week after a depression in the C.G. titre had been noted. Oestrogen therapy in gradually increasing dosage was given from the twentieth week onwards and it reduced the C.G. level to 10,000 I.U. at the twenty-sixth week. After that, however, while still on oestrogen therapy readings climbed steeply. Apparently the patient had become quite "refractory" to stilboestrol in the dosage given. Accordingly, the drug was stopped at the thirty-first week. On restarting at the thirty-second week, slight depression of the reading was obtained with a smaller dosage than that previously employed. This probably indicated the re-establishment of some degree of sensitivity. (Fig.12). Although the C.G. levels in this patient were extremely high throughout pregnancy, the weight of the baby at birth was not abnormal. (See Table 11).

TABLE 11/

TABLE 11

C.G. Readings in Pregnant Diabetic

Name of Patient - Mrs. M. Ba.

Week of Pregnancy	C.G. I.U. per 24-hours	Stilboestrol
10	19,250	
11	(20,530 (24,290	
12	(65,780 (61,490 (58,190	
13	(39,476 (41,110	
14	41,130	
15	(42,300 (41,800	
16	(23,580 (26,770	25mg. per day
17	(28,020 (21,807	30mg. per day
18	24,940	Stop stilboestrol
19	(26,140 (27,130	20mg. per day
20	(28,000 (17,800	"
21	16,980	"
22	15,980	"
23	13,130	"
24/		

TABLE 11 - Contd:- (Patient - Mrs. M. Ba.)

Week of Pregnancy	C.G. I.U. per 24-hours	Stilboestrol
24	14,160	20mg. per day
25	16,740	30mg. per day
26	(12,110 (10,080	"
27	12,650	"
28	17,590	"
29	21,680	"
30	(37,600 (33,130	40mg. per day
31	35,300	(strol. Stop stilboe-
32	39,000	15mg. per day
33	(30,930 (30,000	20 30mg. per day
34	28,930	30mg. per day
35	28,880	"
36	(26,360 (24,760	" "

Case VII - Mrs. L.M. Age 23.

This was the patient's first pregnancy and she was first seen at the thirtieth week. Diabetes had been present for 9½ years.

She developed a moderate hydramnios at the thirty-second week. Very slight ankle oedema and a transient albuminuria were noted at this stage. The child was a breech presentation. At thirty-three weeks the membranes ruptured causing a leak of amniotic fluid.

The diet given was 2,800 calories with 235g. of carbohydrate. Blood sugar figures were never very high and were usually below 180mg. per cent at noon. The renal threshold was very low - 60-70mg.%. Insulin requirements gradually rose from 60 Units at twenty-six weeks to 70 Units at thirty-six weeks.

Delivery occurred at thirty-six weeks. It was spontaneous and by the vaginal route. The labour was difficult and prolonged, lasting thirty-six hours. The head was extracted by forceps and an episiotomy was necessary. Puerperal infection commenced/

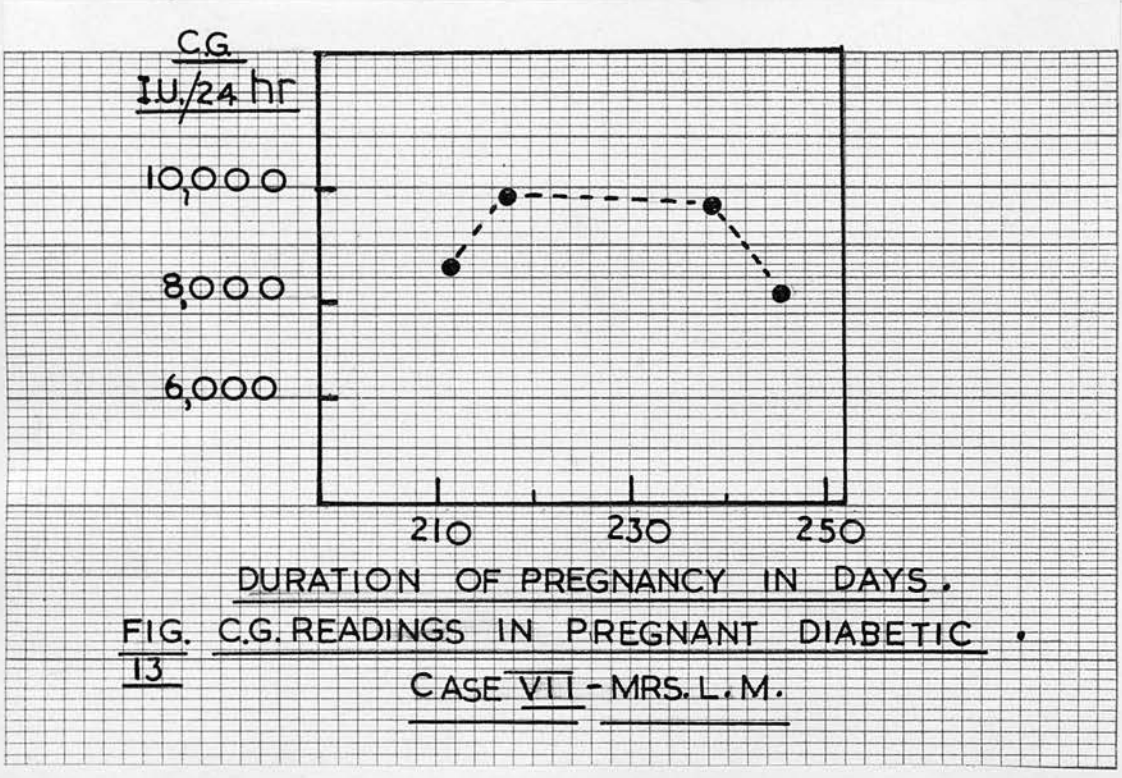

FIG. 13 C.G. READINGS IN PREGNANT DIABETIC. CASE VII - MRS. L. M.

commenced 24-hours after delivery but was controlled by penicillin and sulphonamides. Dienestrol was given to suppress lactation.

The baby weighed 6lbs. 5oz. at birth and the placenta 1lb. 5oz. The infant was shocked, respiration being very feeble. It was not oedematous or unduly large, but it died two days after birth from respiratory failure. On post-mortem examination bilateral suprarenal haemorrhage was found. The pancreatic islets showed marked hypertrophy.

Comment: The death of this baby must be attributed to the diabetes at least in part, although the difficult labour was no doubt a contributory factor.

C.G. Readings:- The four estimations made during the last six weeks of pregnancy were within normal limits. No stilboestrol was given. (Fig.13 and Table 12).

TABLE 12/

TABLE 12

C.G. Readings in Pregnant Diabetic

Name of Patient - Mrs. L.M.

Week of Pregnancy	C.G. I.U. per 24 hours	Stilboestrol
30	8,680	None given
31	10,000	
34	9,980	
35	8,170	

Case VIII - Mrs. B.B. Age 24.

Previous history:- The patient was a primipara. During the present pregnancy she was first seen at the eleventh week. At this time, diabetes had been present for eight years. There was no family history of the disease.

The patient showed no retinal, cardiovascular or neurological complications of diabetes. She had no hypoglycaemic attacks during pregnancy. A severe ketosis developed at the thirty-first week requiring large dosage of insulin. At thirty-three weeks a moderate degree of pre-eclampsia was observed but this was not progressive in type. The diet given during pregnancy almost amounted to a free diet with over 200g. of carbohydrate per day. Insulin requirements rose steadily during pregnancy. At the eleventh week the patient required 80 Units per day and at the twenty-eighth week, 104 Units per day.

Delivery was by Caesarean section at the thirty-fourth week. The operation was uneventful. The baby weighed 6lb. 3½oz. and the placenta 1lb. 5oz. The latter showed numerous infarcts. The baby was a flabby/

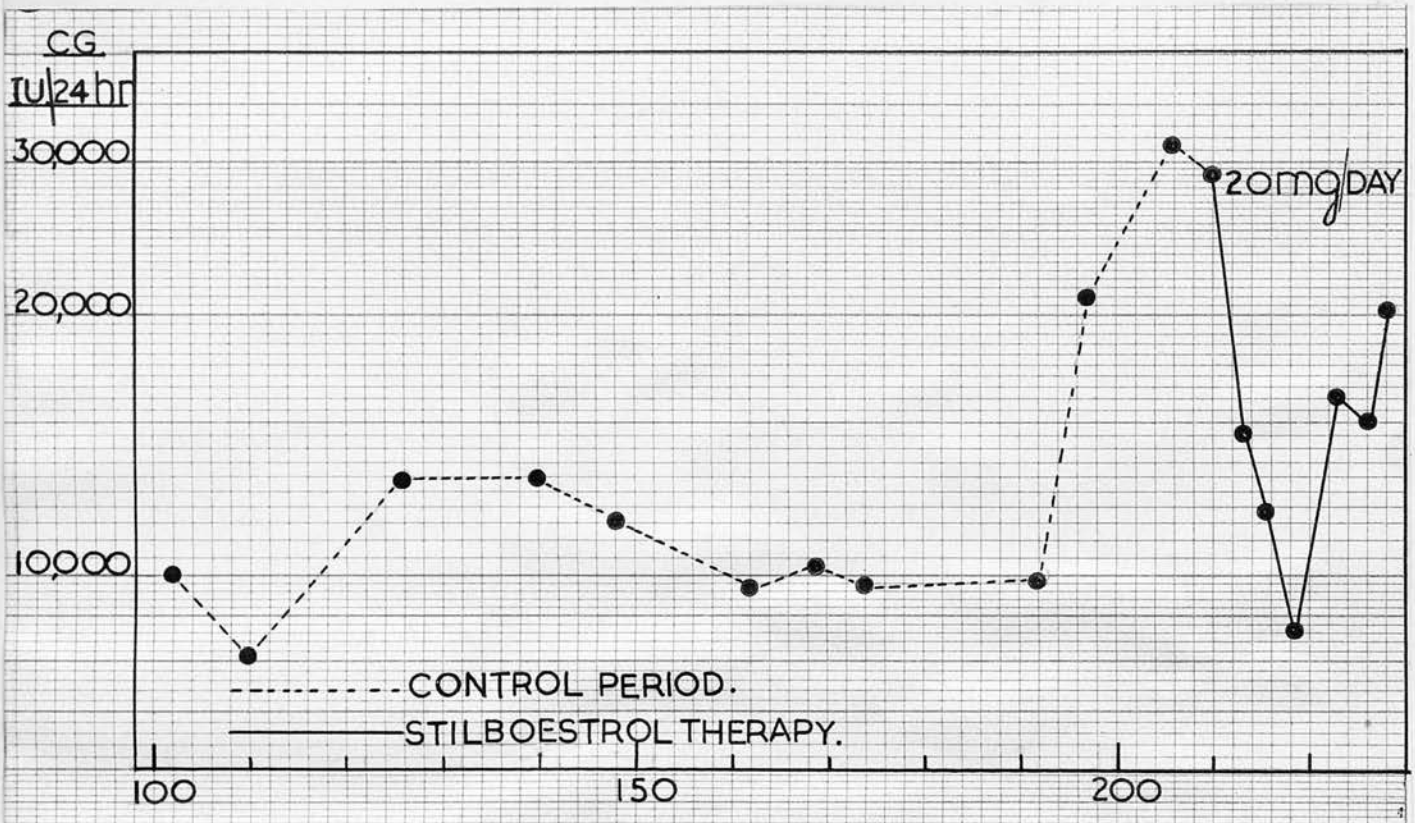

FIG. 14. C.G. READINGS IN PREGNANT DIABETIC TREATED WITH STILBOESTROL.

CASE VIII — MRS B.B.

flabby oedematous infant. Respirations were feeble initially, but the child made gradual progress and was discharged from hospital in a satisfactory condition.

C.G. Readings:- Estimations were made on this patient at approximately weekly intervals from the fourteenth week onwards. The level tended to fluctuate somewhat both above and below the 10,000 I.U. level. At the beginning of the twenty-eighth week, however, a very sharp rise occurred, the figure climbing rapidly to 30,000 I.U. per 24-hours. Subsequent therapy by stilboestrol in dosage of 20mg. per day produced a dramatic fall in the titre but again an "escape" phenomenon occurred when a constant dosage was employed. The results are shown in Fig.14 and Table 13.

Comment: This was the only patient of the series in whom pre-eclampsia was at all marked. The onset of pre-eclamptic symptoms occurred five weeks after the rise in C.G. levels. The baby must be considered to be abnormally heavy for the time of gestation.

TABLE 13/

TABLE 13

C.G. Readings in Pregnant Diabetic

Name of Patient - Mrs. B.B.

Week of pregnancy.	C.G. I.U. per 24-hours	Stilboestrol
14	10,050	
16	8,120	
18	13,000	
20	13,050	
21	11,790	
23	9,750	
24	10,160	
25	9,570	
27	9,850	
28	20,875	
29	30,820	
30	(28,810 (14,570 (11,720	20mg.per day
31	8,600	"
32	16,720	"
33	(15,400 (20,070	"

Case IX - Mrs. C.T. Age 29.

Previous obstetric history: This patient was para 2. (a) 1945: Male child, alive and well.

(b) 1946: Stillbirth at 38 weeks.

During the present pregnancy she was first seen at the tenth week. Diabetes had been present for seven years but there was no family history of the disease. The condition was well controlled during pregnancy there being no ketosis and only a few mild hypoglycaemic episodes.

No hydramnios or toxaemia occurred during the pregnancy. The diet given consisted of 2,500 calories with 200 g. of carbohydrate. Insulin requirements rose gradually during the course of gestation.

This patient had an intra-uterine death at thirty-five weeks followed by a medical induction. The baby weighed 7lb. 14oz. and its length was twenty inches. It showed severe maceration. The placental weight was 1lb. 15oz.

The puerperium was uneventful.

C.G. Readings: This patient was carefully studied from the sixteenth week onwards. Nineteen readings in/

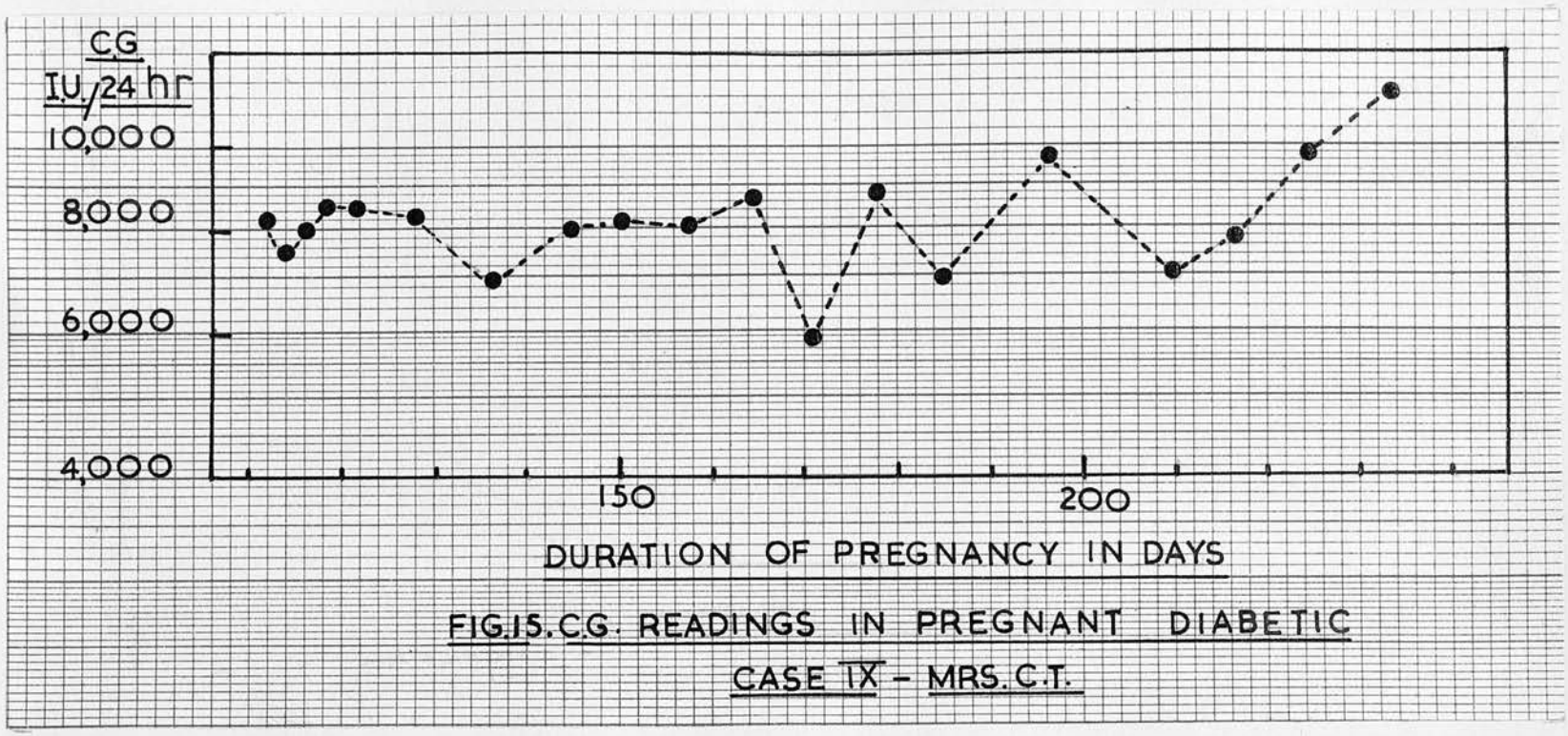

FIG.15. C.G. READINGS IN PREGNANT DIABETIC CASE IX - MRS. C.T.

in all being obtained. The figures were very constant indeed throughout pregnancy, the degree of variation being much less than in the majority of patients. Only the last reading of 11,750 at the thirty-fourth week was at all abnormal. In spite of this, however, the patient had an intra-uterine death at the thirty-fourth week. Cases like this must go far to disprove the theory of White that one can forecast impending obstetrical disasters by a rise in the C.G. titre. (See Fig.15 and Table 14). There was no indication for stilboestrol in this patient and no hormonal therapy was given.

TABLE 14/

TABLE 14

C.G. Readings in Pregnant Diabetic

Name of Patient - Mrs. C.T.

Week of Pregnancy.	C.G. I.U. per 24-hours	Stilboestrol
16	(8,150 (7,700 (8,060	None given
17	(8,580 (8,576	
18	8,200	
19	6,850	
20	8,000	
21	8,220	
22	8,150	
23	8,730	
24	5,856	
25	8,960	
26	6,945	
28	9,700	
30	7,040	
31	7,840	
32	9,930	
34	11,750	

Case X - Mrs. J.U. Age 40

Previous obstetric history: This patient was para 3.

(a) 1930: Fulltime baby: spontaneous delivery: child alive and well.

(b) 1932: Fulltime baby: spontaneous delivery: child alive and well.

(c) 1935: Fulltime baby: spontaneous delivery: child alive and well.

Diabetes developed during the present pregnancy in which the patient was first seen at the twentieth week. The blood sugar curve showed a raised fasting level with delayed return to the fasting level. There was considerable glycosuria during pregnancy. No insulin was required, the patient being treated by diet alone. She was given 2,000 calories with 180g. of carbohydrate.

The patient showed no hydramnios and no toxaemia. Hypertension developed at the twenty-sixth week, the readings being 180/100 at this time. This was thought to be a manifestation of essential hypertension and was not considered to be due to pregnancy toxaemia as there was no oedema and no albuminuria. A renal function test (urea concentration/

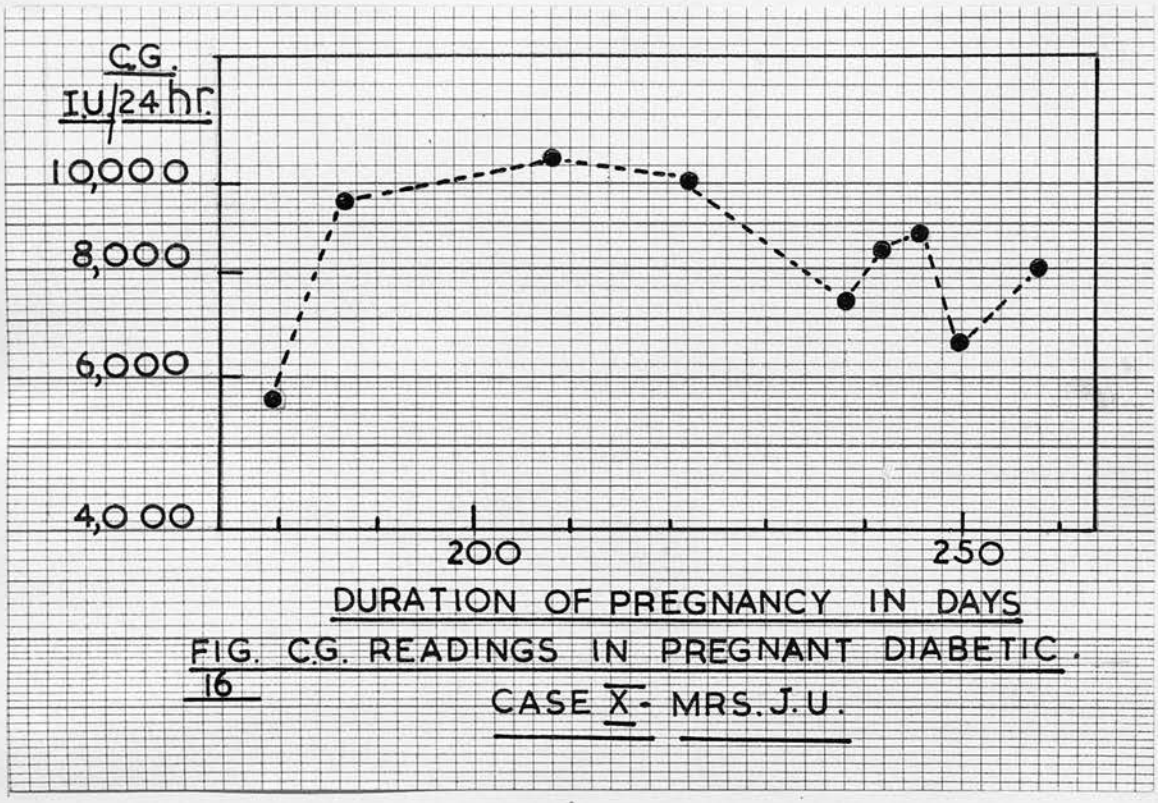

FIG. 16 C.G. READINGS IN PREGNANT DIABETIC. CASE X - MRS. J.U.

concentration range) was normal and the morning S.G. of the urine was satisfactory.

Delivery was by Caesarean section at the thirty-seventh week. Sterilisation was performed at the same time. The weight of the baby was 6lb. 13¼oz. and that of the placenta 1lb. The puerperium was uneventful and mother and child were discharged on the tenth day. The condition of both was satisfactory.

C.G. Readings: Nine readings were obtained on this patient from the twenty-sixth week onwards. Two readings, one at the twenty-ninth and another at the thirty-second week, were just above 10,000 I.U. per 24-hours, but subsequently, the readings fell to normal levels. No hormonal treatment was given. (See Fig.16 and Table 15).

TABLE 15/

TABLE 15

C.G. Readings in Pregnant Diabetic

Name of Patient - Mrs. J.U.

Week of Pregnancy	C.G. I.U. per 24-hours	Stilboestrol
26	(5,643 (9,790	None given
29	10,870	
32	10,140	
34	7,430	
35	8,470	
36	8,808	
37	6,760	
38	8,121	

Case XI - Mrs. J.Ph. Age 30.

Previous obstetric history: This patient had a stillbirth at thirty-nine weeks in 1946. During the present pregnancy she was first seen at the sixteenth week. She was a known diabetic, the disease having commenced in September, 46. There was no family history of the condition. She showed a mild, persistent ketosis from the thirty-fourth week, it being impossible to rid her of acetone due to the low renal threshold. There were slight hypoglycaemic attacks during pregnancy consisting of occasional clouding of vision and slight headache.

Pre-eclampsia did not occur but hydramnios was marked from the thirty-second week. There was no vomiting. Renal threshold was very low, circa 90mg. per cent. Insulin requirements gradually rose during pregnancy.

Delivery was by Caesarean section at the thirty-sixth week. The operation was uneventful. The baby, a female, weighed 9lb. 15¾oz. at birth showing some degree of oedema which was soon evacuated. The placental weight was 2lbs. The puerperium/

puerperium was uneventful and the child throve well. It was subsequently learned that the infant had developed gastro-enteritis from which it recovered.

C.G. Readings: Stilboestrol, in dosage of 30mg. per day, was given to this patient at the twentieth week of pregnancy for experimental purposes although the C.G. readings were not high. A depression was produced, the titre falling from approximately 10,000 I.U. per 24-hours to 7,000 I.U. and rising slightly when the drug was stopped. Starting from the twenty-first week of pregnancy, stilboestrol was given in gradually increasing dosage each week. This was done on an empirical basis. Two readings were made at the twenty-fourth week when the patient was receiving 40mg. of stilboestrol per day. The figures obtained were 14,000 I.U. per 24-hours in the two specimens and were much higher than anticipated, considering the dosage of oestrogen employed. No further estimations were undertaken till the beginning of the thirty-second week of pregnancy and although by this time the patient was/

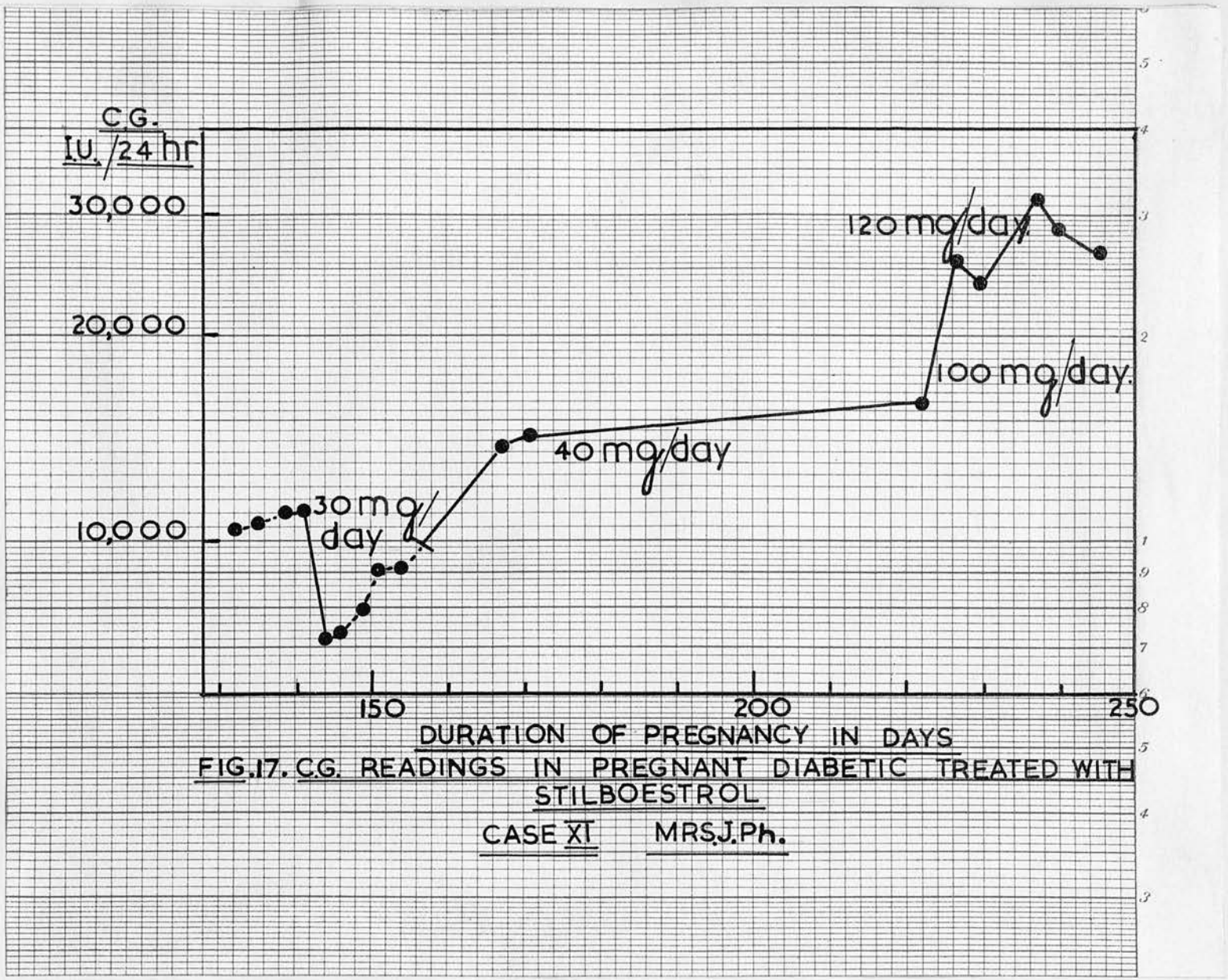

FIG.17. C.G. READINGS IN PREGNANT DIABETIC TREATED WITH STILBOESTROL

CASE XI MRS.J.Ph.

was receiving massive dosage of oestrogen (100mg. of stilboestrol per day), the readings were very high indeed, being well over 20,000 I.U. per 24-hours. They remained very high until delivery at thirty-six weeks even although the oral stilboestrol was supplemented by 100mg. per day of oestradiol benzoate and 30mg. per day of pure oestradiol in the last three weeks of gestation.

It appeared, therefore, that this patient, who was originally sensitive to stilboestrol, became completely refractory to massive dosage of oestrogen in the later stages of pregnancy. The marked degree of hydramnios at the time of delivery was thought to be associated with the prolonged treatment by oestrogen, the water-retaining power of oestrogen having been demonstrated previously by numerous workers.

This was one of the few cases in whom the hyperprolanuria was associated with an unduly large baby for the time of delivery and an unduly large placenta. (See Fig.17 and Table 16).

TABLE 16/

TABLE 16

C.G. Readings in Pregnant Diabetic

Name of Patient - Mrs.J.Ph.

Week of Pregnancy	C.G. I.U. per 24-hours	Stilboestrol
19	(10,400 (10,600 (11,000	-
20	(11,000 (7,100 (7,200	30mg. per day
21	(8,000 (9,100 (9,100	-
24	(14,000 (14,000	40mg. per day
32	16,280	100mg. per day
33	(26,000 (24,500	100mg.+100mg. oestradiol benzoate. I.M.
34	32,000	"
35	(28,400 (27,180	(100mg. stil- (boestrol (120) (100mg. oestrad. (benzoate, I.M. (20mg. pure (oestradiol.

Case XII - Mrs. A.R. Age 40

Previous obstetric history: This patient was para 6.

(a) 1931: A fulltime male baby, weighing 10½lbs: delivery spontaneous: child alive and well: pregnancy normal.

(b) 1931: A fulltime female baby, weighing 10lbs: spontaneous delivery and child is alive and well: pregnancy normal.

(c) 1938: A fulltime male infant which died at birth. The cause of death was not ascertained. Pregnancy was normal and delivery spontaneous.

(d) 1939: A fulltime male baby, weighing 10½lbs. The child is alive and well: pregnancy normal and delivery spontaneous.

(e) 1941: Fulltime male baby which died at birth. The cause of the death was not known.

(f) 1943: A fulltime male infant which also died at birth from unknown causes. As before, the pregnancy and delivery were normal.

In the present pregnancy the patient was first seen at the sixteenth week. There was no family history of diabetes, the condition having been present in the patient for five years.

Insulin/

Insulin requirements during pregnancy were small rising slightly as term approached. At thirty-six weeks she was taking 16 Units per day, (8 Z.P. and 8 soluble).

During her pregnancy she had no toxaemia and no vomiting. At the twenty-sixth week she was admitted to Simpson Maternity Pavilion as a threatened abortion, having had a very small haemorrhage two days prior to admission. There was no further bleeding and she was discharged from hospital after ten days. The remainder of her pregnancy was uneventful.

Delivery was by Caesarean section at the thirty-eighth week. Sterilisation was undertaken at the same time. The baby weighed 10lb, the child being the typical large, oedematous infant of the diabetic. The placental weight was 2lb 3½oz. The puerperium was uneventful and mother and child left hospital in good condition after eleven days.

C.G. Readings: Only five estimations were made on this patient during her pregnancy. Of these, three were well below 10,000 I.U. and the two prior/

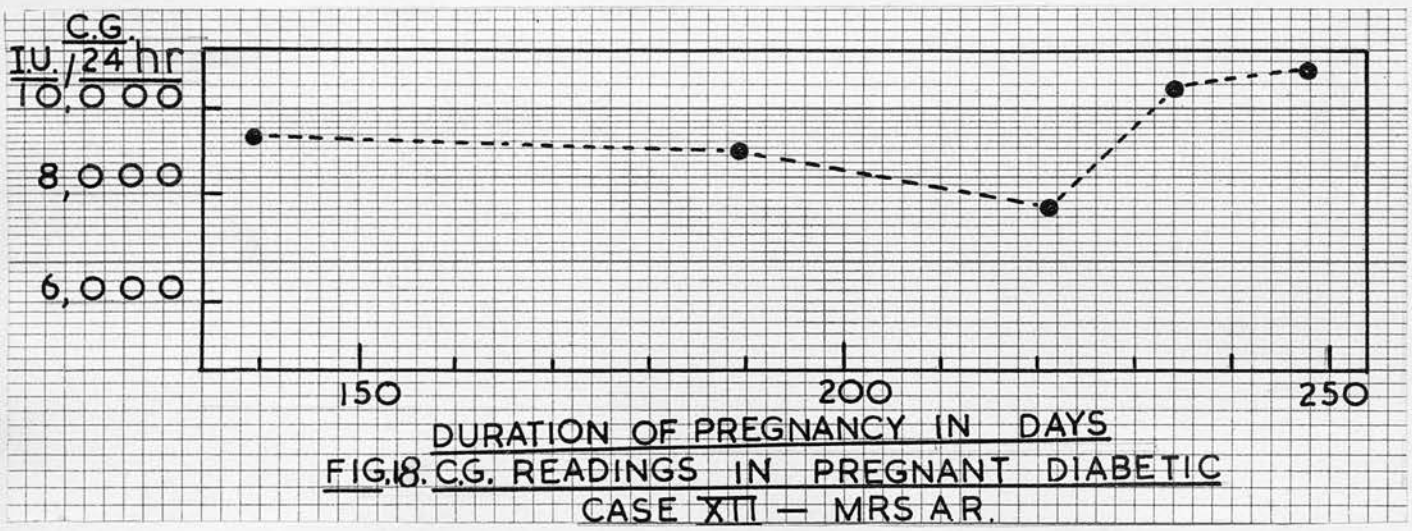

FIG.18. C.G. READINGS IN PREGNANT DIABETIC CASE XII — MRS A.R.

prior to delivery, just above 10,000 I.U. The baby was the heaviest of the series of cases studied, weighing 10lb. at birth and the placenta was also unduly large. There was, therefore, no correlation between C.G. readings on the one hand and weight of baby and placenta on the other. (Fig. 18 and Table 17). No stilboestrol was given. In the previous obstetric history, it was noted that this patient had delivered at least three children of 10lb. weight or over.

TABLE 17/

TABLE 17

C.G. Readings in Pregnant Diabetic

Name of Patient - Mrs. A.R.

Week of Pregnancy	C.G. I.U. per 24-hours	Stilboestrol
20	9,330	None given.
27	8,702	
31	7,460	
32	11,090	
36	11,320	

Case XIII - Mrs. J. Pa. Age 27

Previous obstetric history: This patient was para 2, both stillbirths.

(a) 1941: At thirty weeks she had a premature labour, giving birth to a stillborn male child.

(b) 1943: Intra-uterine death at thirty-five weeks.

In her present pregnancy the patient was first seen at the twelfth week. Diabetes had been present for six years. There was no family history of the disease.

Insulin requirements rose steadily during pregnancy and at the thirty-sixth week she was taking 126 Units per day. The diet consisted of 2,600 calories with 200g. of carbohydrate. Blood sugars were very variable throughout pregnancy.

She developed a slight degree of ankle oedema at the thirty-fifth week but there was no hypertension and no albuminuria.

Delivery was by Caesarean section at the thirty-seventh week. The baby weighed 6lb. 7¾oz. and the placenta 1lb. 6oz. The child throve well and was discharged from hospital in good condition./

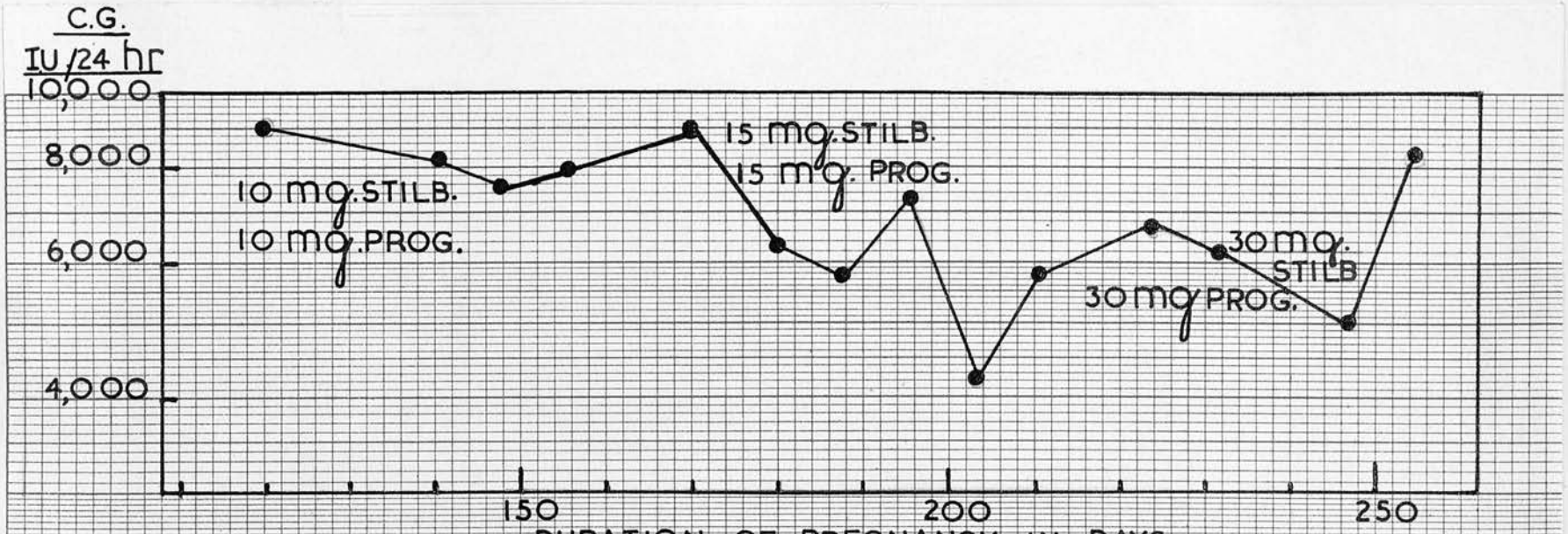

FIG. 19. C.G. READINGS IN PREGNANT DIABETIC CASE XIII MRS JESSIE Pa.

THIS PATIENT WAS GIVEN STILBOESTROL AND PROGESTERONE THROUGHOUT PREGNANCY ON AN EMPIRICAL BASIS.

condition. The mother developed a local uterine infection on the third day of the puerperium and this was successfully treated by penicillin and sulphonamides.

C.G. Readings: This was the only patient in the series who was given combined therapy with oestrogen and progesterone throughout her pregnancy. Unfortunately, no readings were obtained before the start of oestrogen therapy. The hormonal treatment was given on an entirely empirical basis. Fourteen readings in all were obtained on this patient, all of which were entirely normal. From comparison of findings in previous cases given oestrogen therapy on the basis of a high C.G. reading , it is concluded that in this case, the reading would have been normal in the absence of hormonal therapy which did not materially affect the results. At the end of pregnancy, this patient was receiving 30mg. of oestrogen and 20mg. of progesterone per day. (Fig. 19 and Table 18).

TABLE 18/

TABLE 18

C.G. Readings in Pregnant Diabetic

Name of Patient - Mrs. J.Pa.

Week of Pregnancy	C.G. I.U. per 24-hours	Stilboestrol
17	9,000	10mg.per day + 10mg.Progesterone I.M. per day.
20	8,200	"
21	7,600	"
22	8,000	"
24	9,000	15mg. per day + 15mg.Progesterone I.M. per day.
25	6,400	"
26	5,800	"
27	7,500	"
29	4,200	"
30	5,900	"
32	6,600	"
33	6,400	30mg. per day + 30mg. Progesterone I.M. per day.
35	5,600	"
36	8,500	"

Case XIV - Mrs. M.C. Age 41.

Previous obstetric history: This patient was para 2 with 2 abortions.

(a) 1943: After a long labour, a fulltime child weighing 9lb. 4oz was stillborn.

(b) 1944: Baby was fulltime, but again, stillborn; birth weight 7lbs.

(c) 1946: Abortion at 2 months.

(d) 1947: Abortion at 2 months.

During the present pregnancy the patient was first seen at the thirty-fourth week. Diabetes was first noted during this pregnancy. There was no family history of the disease. No insulin was required, the condition being adequately controlled by dietetic measures alone. Actually, the blood sugar curve in this case, was of a very "borderline" type, and it was only after it had been repeated on three occasions that the patient was definitely classified as a diabetic. There was considerable glycosuria but no ketosis.

The patient had no oedema, hypertension and albuminuria and hydramnios was absent. Delivery was/

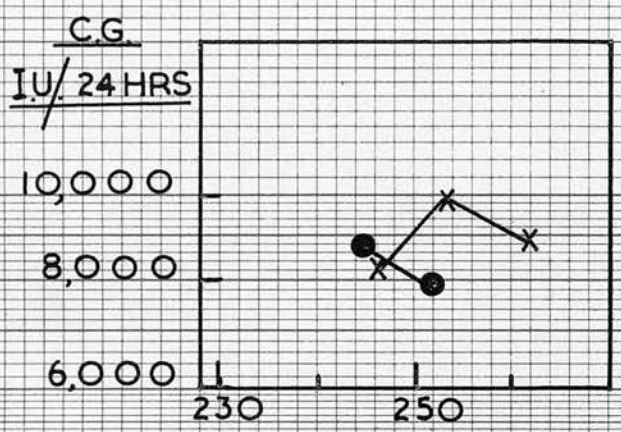

FIG. 20 - CG READINGS IN 2 PREGNANT DIABETICS

●——● - MRS. M.C.

X——X - MRS. J.A.

was by Caesarean section at the thirty-seventh week. The operation was uneventful, the weight of the child 7lb. 6¾oz. and of the placenta 1lb. 5oz. At the end of the puerperium the condition of both mother and child was satisfactory.

C.G. Readings: Only two readings were obtained on this patient, one at the thirty-fifth and one at the thirty-sixth week. In both cases, the titre was normal. (Fig.20 and Table 19).

Comment: Like Case V and X this patient was the older obese type of diabetic in whom the condition was relatively mild, and in whom insulin was not required. The bad obstetric history was noted.

TABLE 19

C.G. Readings in Pregnant Diabetic

Name of Patient - Mrs. M.C.

Week of Pregnancy	C.G. I.U. per 24-hours	Stilboestrol
35	8,600	Not given
36	7,800	

Case XV - Mrs. J.A. Age 29

Previous obstetric history: This patient was para 1 with an abortion.

(a) 1945: At 20 weeks she had a spontaneous abortion.

(b) 1946: She had a fulltime female infant weighing 7lb. 12½oz. The child is alive and well.

During the present pregnancy the patient was first seen at the twentieth week. Diabetes had been present for 5½ years. There was no family history of the condition. Insulin requirements during pregnancy showed a steady rise. At the thirty-sixth week she was receiving 74 Units (Z.P. + soluble) per day.

There was no evidence of pre-eclampsia and no hydramnios during the period of gestation. At the thirty-sixth week she fell on the way to the X-ray department and was admitted to hospital for observation. The foetal heart became very faint. A medical induction was immediately undertaken but the baby was stillborn. It weighed 8lb. 9oz. and the placental weight was 1lb. 2oz.

In/

In the puerperium the patient developed a local uterine infection which was successfully treated by penicillin and sulphadiazine. Her condition on discharge was satisfactory.

C.G. Readings: Again, only a small number of estimations - three in all - were made from the thirty-fifth to the thirty-seventh week of pregnancy. They were all within normal limits. (Fig.20 and Table 20).

TABLE 20.

C.G. Readings in Pregnant Diabetic

Name of Patient - Mrs. J.A.

Week of Pregnancy	C.G. I.U. per 24-hours	Stilboestrol
35	8,200	Not given.
36	9,800	
37	8,800	

Case XVI - Mrs. M.P. Age 28

This patient was originally classified as a case of diabetes mellitus and had been considered as such during a previous pregnancy. Repeated blood sugar estimations, however, did not confirm the diagnosis, for although the patient had considerable glycosuria during pregnancy, the fasting blood sugar was never above 100mg. per cent and the reading in the glucose tolerance test came back to the fasting level within two hours. This patient could not, however, be classed as a normal pregnancy, firstly because of the considerable glycosuria during pregnancy and secondly, because of the fact that she delivered a 10lb. baby at thirty-eight weeks. It was considered that she should be placed in the category of a pre-diabetic.

Previous obstetric history: The patient was para 1.

1947: The child was a fulltime baby weighing/

weighing 7lb. 2oz. It is alive and well; delivery was spontaneous.

In the present pregnancy, the patient was first seen at the fifteenth week. No insulin was required during pregnancy. The noon blood sugars were relatively constant, the highest reading obtained being 120mg. per cent.

There were no complications during the pregnancy. A medical induction was undertaken at the thirty-eighth week. A constriction ring developed round the neck of the foetus which was a breech presentation. Manual rotation, using mid-cavity forceps was attempted but eventually the body was delivered by strong continuous traction. The foetus was stillborn and very macerated. Its weight was 10lb. and the placental weight was 1lb. 1loz. Post-mortem examination of the foetus showed two large haematomata on the anterior surface of the liver. These had ruptured with a subsequent haemo-peritoneum.

C.G. Readings: Twelve readings were obtained

in/

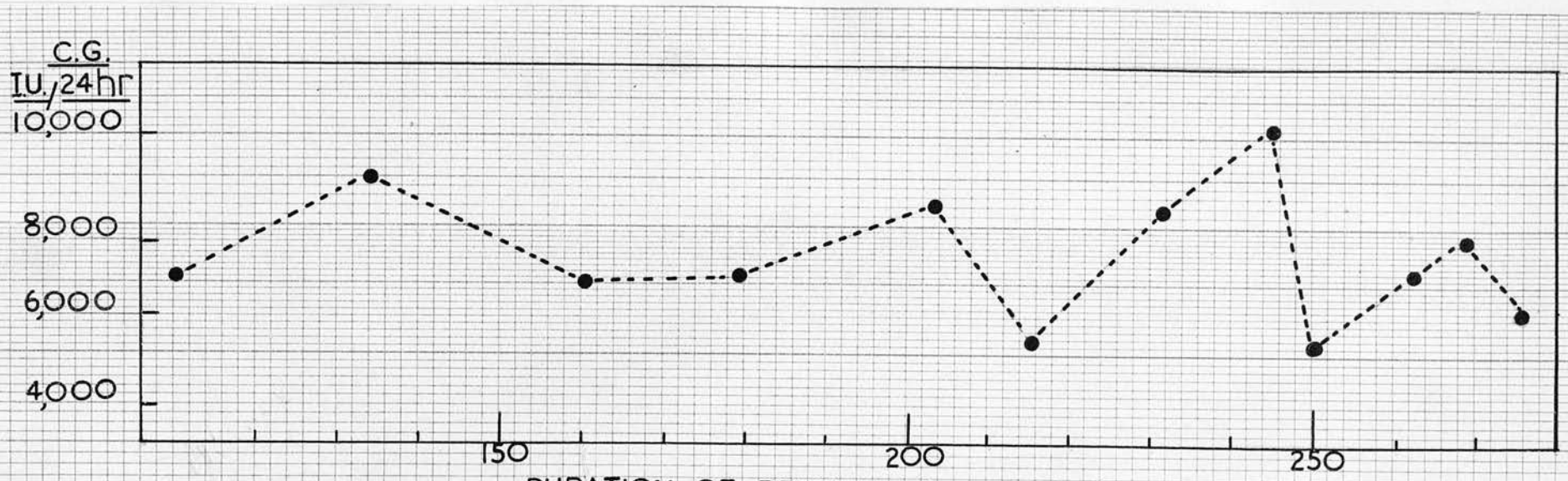

FIG. 21. C.G. READINGS IN PREDIABETIC PREGNANCY
CASE XVI — MRS. M. P.

in this case, all of which were within normal limits. Again, although the patient delivered a very big baby, the C.G. readings were not abnormally high. (Fig.21 and Table 21).

TABLE 21

C.G. Readings in "Pre-diabetic" Pregnancy.

Name of Patient - Mrs. M.P.

Week of Pregnancy	C.G. I.U. per 24-hours	Stilboestrol
15	7,032	Not given
19	10,200	
23	6,800	
27	7,040	
29	8,750	
31	5,750	
33	8,632	
35	10,300	
36	5,360	
37	7,440	
(38	8,220	
(38	6,190	

A summary of the findings in the various cases is shown in Table 22.

TABLE 22

Correlation between C.G. Readings and Medical and Obstetrical findings in Pregnant Diabetics.

CASE NO.	PATIENT	Time of Delivery (weeks)	Mode of Delivery	Placental weight	Weight of baby.	Subsequent fate of baby.	C.G. Readings during pregnancy.	Hormonal therapy.	Insulin requirements during pregnancy.	Signs of pregnancy toxaemia.
I	O.B.	38	Vaginal	1lb. 8oz.	6lb. 15½oz.	Viable	Normal throughout.	None given	Gradual rise.	None
II	M.T.	36	Caesarean section.	1lb.10oz.	6lb. 7oz.	Viable	Abnormally high from 18th week.	Stilboestrol.	Gradual rise.	None
III	H.E.	38	Vaginal	1lb. 5oz.	7lb. 1½oz.	Viable	Normal throughout.	None given	Gradual rise to 33rd week	None
IV	C.B.	36	Vaginal	1lb. 3oz.	4lb. 11oz.	Stillborn.	Abnormally high from 25th week.	Stilboestrol.	Gradual rise.	None
V	M.Be.	41	Caesarean section.	1lb.15oz.	9lbs.	Viable	Abnormally high from 24th week.	Stilboestrol.	No insulin given.	None
VI	M.Ba	37	Caesarean section.	1lb. 2oz.	6lb. 1oz.	Died after 1 wk.	Abnormally high from 10th week.	Stilboestrol.	Steady rise.	None
VII	L.M.	36	Vaginal	1lb. 5oz.	6lb. 5oz.	Stillborn.	Normal (4 readings.)	None given	Gradual rise.	Very slight hypertension.
VIII	B.B.	34	Caesarean section.	1lb. 5oz.	6lb. 3½oz.	Viable	Abnormally high from 28th week.	Stilboestrol.	Steady rise.	Hypertension at 32 weeks.
IX	C.T.	36	Vaginal	1lb.15oz.	7lb. 14oz.	Intrauterine death.	Normal throughout.	None given	Gradual rise.	None
X	J.U.	37	Caesarean section.	1lb.	6lb.13¼oz.	Viable	Normal throughout.	None given	No insulin required.	None

XI/

TABLE 22.(contd.)

CASE NO.	PATIENT	Time of Delivery (weeks)	Mode of Delivery	Placental weight.	Weight of baby.	Subsequent fate of baby.	C.G. Readings during pregnancy.	Hormonal therapy	Insulin requirements during pregnancy.	Signs of pregnancy toxaemia.
XI	J.Ph.	36	Caesarean section.	2lbs.	9lbs.15¾oz.	Viable	Abnormally high from 20th week.	Stilboestrol Oestradiol Benzoate. Pure oestradiol.	Steady rise.	None
XII	A.R.	38	Caesarean section.	2lb. 3⅛oz.	10lbs.	Viable	Normal throughout.	None given	Slight rise.	None
XIII	J.Pa.	37	Caesarean section.	11b. 6oz.	61b. 7¾oz.	Viable	Normal throughout.	Stilboestrol Progesterone throughout pregnancy.	Steady rise.	None
XIV	M.C.	37	Caesarean section.	11b. 5oz.	71b. 6¾oz.	Viable	Normal (2 readings).	None given.	No insulin required.	None
XV	J.A.	37	Vaginal	11b. 2oz.	81b. 9oz.	Stillborn.	Normal (3 readings).	None given.	Steady rise.	None
XVI	M.P.	39	Vaginal	11b. 11oz.	10lbs.	Stillborn.	Normal throughout.	None given.	No insulin required.	None

DISCUSSION.

In the investigation of this subject there are really two distinct problems involved. Firstly, one must consider the C.G. readings in these patients, and the effect of stilboestrol on these readings, and secondly, there is the question of whether stilboestrol actually is of value in reducing the foetal loss rate in diabetic pregnancies. These considerations will be discussed in turn.

From a study of the C.G. readings in the above patients, it is apparent that a proportion of diabetic women show an abnormally high excretion of C.G. as compared with the normal cases discussed in a previous section. Of the cases dealt with, six out of fifteen, or 40.0% showed consistently high figures for C.G. excretion. In the remaining ten cases, the vast majority of the readings were below 10,000 I.U. per 24-hours. In Case I, however, a reading, just before delivery, of/

of 13,000 I.U. per 24-hours was obtained. This was thought to be of relatively little significance as all the previous readings had been perfectly normal. Similarly, in Case XII, readings obtained in the thirty-second and thirty-sixth weeks of pregnancy were above 11,000 I.U. per 24-hours, but readings were made too infrequently on this patient to place her definitely in the "high C.G." category. Case IX also showed one abnormal reading just prior to delivery.

The time of pregnancy at which the rise in C.G. levels occurred varied considerably with individual patients. In Case II, (Table 7), readings were usually high when the patient was first seen at the eighteenth week of pregnancy. In Case IV, (Table 9), a slight elevation in the level was noted at the twenty-seventh week, and it gradually climbed to 20,850 I.U. by the thirty-second week. In Case V, (Table 10), the readings were normal when the patient was seen at the twentieth week. After that some lability of the readings was observed and this was followed by a rapid rise to over 20,000 I.U. per 24-hours at the twenty-eighth week of pregnancy. Case VI, (Table 11), presented

a/

a very difficult endocrinological problem. This patient was seen at a much earlier stage of pregnancy than the majority of the other diabetics and it was thought at first that the very high readings obtained from the eleventh to the thirteenth week merely represented the normal "peak period", slightly delayed. However, when the readings remained consistently high in the fourteenth and fifteenth weeks it was concluded that the elevation was pathological and this was borne out by subsequent findings. The readings in Case VIII, (Table 13), showed some lability in the earlier stages, similar to that noted in Case V and here too, a sudden increase occurred, this time at the twenty-ninth week of pregnancy. In Case XI, (Table 16), readings were normal when this patient was first examined at the nineteenth week, but at the twenty-fourth week were much higher than was expected since oestrogen was being administered.

In her series of cases, White (1946) reported/

reported "hormonal imbalance" in 80% of her patients, whereas in the cases studied in Edinburgh, the abnormality was found in only 40%. However, the number of cases studied by White (271 in the course of ten years) is so very much larger than the present series that no valid comparison can be made. Mention must be made, however, of the method of assay of C.G. used by White in this study. C.G. was estimated in the serum. The latter was precipitated with ethanol and allowed to stand overnight. It was then extracted with ether and the residue suspended in 6 c.c. normal saline, (Zondek's alcohol precipitation method). The quantities of sera used were 0.5, 0.3, 0.2, 0.15 and 0.075 c.c. to give readings of 120, 200, 333, 500, 775, 1,000 and 1,500 rat units per 100 c.c. of serum. Twenty-one day old female rats weighing 35-45g. were injected twice daily for three days and on the morning of the sixth day necropsy was performed. The end-point of the assay depended on the macroscopic determination of corpora lutea in the ovaries. If in the "critical weeks" of pregnancy, i.e., twenty-fourth to thirty-sixth, the/

the results on two occasions exceeded 200 rat units of C.G. per 100 c.c. of serum, oestrogen therapy usually combined with progesterone, was commenced.

There are, however, certain grave disadvantages in this method of biological assay. In the first place, the use of arbitrary "rat units" is always very unsatisfactory and results expressed in terms of the standard preparation of C.G. must be more reliable than those expressed in "units" varying with each laboratory. Secondly, the actual method of assay used depending on corpora lutea, is essentially a subjective test and is therefore liable to variation in interpretation. In addition, the statistical error of the method is very large indeed (Emmens, 1939), and this is bound to cause great inaccuracy in the results. For those two reasons, therefore, the results obtained are open to some degree of criticism.

As in normal women, stilboestrol, when

administered/

administered initially to pregnant diabetics even in relatively small dosage, invariably produced a fall in the C.G. reading. The degree of depression varied from case to case, but tended to be more pronounced in early than in late, pregnancy. This effect is well illustrated by Cases II, IV, V, VIII and XI. On a constant dosage of the oestrogen, however, this depression was not maintained and the reading tended to climb back to its original level while treatment was still being given. This was seen in Cases II, IV, V, VI and VIII, and is best described as an "escape" phenomenon. When this occurred, subsequent increase of dosage in the majority of cases temporarily produced another depression of the reading but this depression was often very evanescent. Certain patients, however, appeared to become completely refractory to stilboestrol. This was very well illustrated by Case XI. This patient was originally sensitive to stilboestrol in early pregnancy. In the last weeks, however, in spite of massive dosage of oestrogens (100mg. of stilboestrol per day plus 100mg. of oestradiol benzoate per day plus/

plus 20mg. pure oestradiol per day), no significant depression of the very high C.G. level occurred. Case VI also demonstrated this refractoriness, no depression of C.G. readings being produced by a dosage of 40mg. per day. There is some evidence, however, as shown in Cases II and VI, that by cessation of the oestrogen therapy for a few days, sensitivity to the drug may be re-established and a depression of the titre may be produced by a dose smaller than that originally employed, (Case VI).

The effect of stilboestrol on the C.G. readings may therefore be considered to have five phases. These are (i) initial depression;

(ii) subsequent escape;

(iii) further depression by an increase in dosage;

(iv) refractoriness after prolonged treatment;

(v) increased sensitivity to the drug on restarting treatment after cessation for a few days.

The mechanism of action of stilboestrol in producing/

producing these effects is far from clear. One of the main tenets of the endocrinological theories of Smith and Smith (1936: 1938: 1941: 1948), regarding the actiology of pre-eclampsia and also of diabetic pregnancy, is that the rise in the C.G. is essentially a "compensatory" phenomenon due to lack of oestrogen and progesterone in these conditions. It is also suggested by these workers that the primary cause of pre-eclampsia is deficient utilisation of C.G. for the production of oestrogen and progesterone and they consider that low or normal figures for the C.G. in diabetic pregnancy may be explained by a considerable assault on the placenta, e.g., by massive infarction causing deficient elaboration of the placental gonadotrophin. The theory that the beneficial effects of oestrogen in diabetic pregnancies is due to its effect in increasing progesterone production has been disproved by the recent work of Sommerville, Marrian and Clayton (1949). These workers found that the administration of stilboestrol actually decreased the quantity of urinary pregnanediol and did/

did not increase it as stated by the Smiths. The views of the Smiths accordingly are no longer tenable.

Studies in the experimental animal have indicated that oestrogen given in the proper dosage will suppress or inhibit the gonadotrophic activity of the anterior pituitary. (Meyer and Biddulph, 1941; Meyer, Biddulph and Finnerty, 1946; Robson, 1948). Moore, (1935), investigated the action of ovarian hormones very thoroughly and also found an inhibitory effect of the ovarian hormone on the pituitary. This worker suggested that a reciprocal relationship existed between the pituitary and the gonads and this might be responsible for the cyclical nature of ovarian function; as the ovarian secretion increases the oestrogen produced by the ovaries inhibits pituitary activity, this in turn, causes a decrease in the stimulation of the ovary by the pituitary and the consequent decrease in ovarian secretion is followed by a cessation of the pituitary inhibition and thus by a resumption of the pituitary secretion. Confirmation of Moore's hypothesis was produced by the experiments of Meyer, Leonard, Hisaw and/

and Martin (1932), who showed that the pituitary of rats injected with oestrogen contained less gonadotrophic hormone than in untreated control animals. Paradoxically, however, there is some evidence due to Hohlweg (1935), and to Halpern and D'Amour (1936), that oestrogen given in a somewhat different schedule of dosage may actually stimulate the release of gonadotrophin from the pituitary and histological evidence is presented in support of this contention. These varying results are probably due to differences in dosage of the oestrogenic preparations used.

In the human, however, the action of oestrogen on the pituitary gonadotrophins appears to be mainly an inhibitory one. Jones and McGregor (1936), showed that the injection of oestrogen into women at the menopause led to a cessation of the increased gonadotrophic hormone excretion in the urine. Albright (1948), found that stilboestrol in dosage of lmg. per day for thirty days depressed the F.S.H. in the urine of menopausal patients from 380 to less than 6.5 mouse units per 24-hours, and Rowlands and Sharpey-Schafer (1940), demonstrated that the administration of oestradiol benzoate decresed the gonadotrophic hormone/

hormone content of the pituitary of post menopausal women.

All this previous work has dealt exclusively with the inhibitory effect of oestrogen on the pituitary gonadotrophins but there appears to be no literature available on the effect of oestrogens in inhibiting the gonadotrophic hormone elaborated by the chorionic villi of the placenta during pregnancy. This effect has been demonstrated (Loraine, 1949) in normal women and in diabetic women.

When an attempt is made to correlate the C.G. readings with the medical and obstetrical findings in these cases and also to assess the effect of stilboestrol in reducing the foetal mortality rate in diabetic pregnancy, few definite conclusions emerge due mainly to the small number of patients so far studied. It will be remembered that Priscilla White in her study of diabetic pregnancies drew four main conclusions. Firstly, only in the group of patients who showed "hormonal imbalance", i.e., high C.G. and low pregnanediol readings did obstetrical accidents and pre-eclampsia occur./

occur. Those patients with normal hormonal readings had a foetal loss rate no higher than normally pregnant women. Secondly, as the rise in C.G. occurred usually some weeks before any obstetrical accident it was thought that this finding could be regarded as the herald of an impending disaster. In the third place, White (1946) stated that only those patients with high C.G. readings delivered unduly large babies and had overweight placentae. Finally, it was believed that a consistent depression of the C.G. readings could be maintained throughout pregnancy by oestrogen therapy which commenced at the twentieth week of pregnancy with 5mg. per day and was gradually increased by 5mg. per week to 30-50mg. per day at the thirty-sixth week. Oestrogens were given either orally or parenterally to these patients.

When these four criteria are applied to the series of cases studied in Edinburgh, many of these conclusions are not confirmed. Of the fifteen patients considered, ten of them had live babies, and a stillbirth or neo natal death occurred in five or 33.3%, (see Table 22). These five patients/

patients were Cases IV, VI, VII, IX and XV. A study of the C.G. readings in these diabetics produces some important evidence. In Case IX, frequent C.G. readings were made from an early stage of pregnancy (Fig. 15 and Table 14), and the figures throughout were remarkably constant. In spite of this, however, the patient had an intra-uterine death at the thirty-sixth week without any previous rise in the C.G. titre. Case VII, had perfectly normal C.G. readings throughout the last eight weeks of pregnancy and yet delivered a stillborn child at the thirty-sixth week. Similarly, in Case XV, although the three C.G. readings made just prior to delivery were quite normal, this patient also had a stillbirth. The position with Cases IV and VI is rather different. It will be remembered that in Case IV the C.G. readings were high when the patient was first seen at the twenty-fourth week and that a transitory depression of the titre was obtained by stilboestrol, the reading eventually climbing back to its original level. It is very doubtful if the foetal death in this case could be attributed to the diabetes per se because of/

of the development of an unfortunate obstetrical complication during labour. In Case VI, the C.G. readings were high throughout the whole pregnancy. Delivery was by Caesarean section at the thirty-seventh week and the child, although healthy when born, died one week later of an aspiration pneumonia. This death must then, to some extent, be attributed to the diabetic condition of the mother. Conversely, certain patients who, in spite of massive oestrogen therapy, had very high C.G. readings just before labour, delivered viable children. This is illustrated by Cases II, V, VIII and XI. In Case II, the C.G. reading just prior to delivery was 13,900 I.U. per 24-hours; in Case V, it was 20,720; in Case VIII it was 20,070 and in Case XI it was 27,180.

From these studies therefore, no evidence can be found in support of the theory that by a rise in C.G. titre one can forecast a subsequent obstetrical accident. It also appears that, in the small number of cases considered, foetal death is just as likely/

likely to occur in these patients with normal C.G. readings as in those with abnormally high figures.

The relationship of the C.G. level to the weight of baby and placenta will now be considered. Six out of the fifteen cases studied showed consistently abnormally high C.G. readings during pregnancy. These were Cases II, IV, V, VI, VIII and XI. Case II was delivered by Caesarean section at the thirty-sixth week and the weight of the baby was 6lb. 7oz. and of the placenta 1lb. 10oz. These weights are slightly above the normal for this stage of gestation, but the child was not unduly oedematous. In Case IV, who had a vaginal delivery at thirty-six weeks and in whom the C.G. readings were abnormally high, the weights of baby and placenta were below normal for the time of gestation (baby 4lb. 11oz: placenta 1lb. 3oz.). Case V delivered a heavy ba by (9lb.) at forty-one weeks. The child was oedematous at birth, but the placental weight (1lb. 5oz.), was not unduly high. In Case VI, where the C.G. readings were very high throughout pregnancy Caesarean section at the thirty/

thirty-seventh week produced a child weighing 6lb. 1oz. this being quite normal at the period of gestation. The weight of the placenta, (11b. 2oz.) was also within normal limits. Case VIII had a 6lb. 3½oz. baby delivered by Caesarean section at thirty-four weeks. This is above the normal weight for this time of gestation. Case XI, who delivered a baby weighing 9lb. 15½oz. at thirty-six weeks, had the heaviest infant of the "high C.G." group of cases. Here too, the placenta was unduly large (2lbs). On the other hand, the largest baby of the series (10lb. at thirty-eight weeks), was delivered by a patient (Case XII) who had normal C.G. readings during pregnancy, showing only a very slight rise to 11,000 I.U. for 24-hours just before delivery. Case XIV, who delivered a 7lb. 6¾oz. baby at thirty-seven weeks and Case XV, who had an 8lb. 9oz. baby also at thirty-seven weeks, both had normal C.G. readings just prior to delivery. Case VII and VIII who also had normal readings during pregnancy delivered unduly large infants at the thirty-sixth week in both cases. Therefore, there appears to be no correlation between a high C.G. reading/

reading on the one hand and increased weight of the baby on the other. Patients with normal C.G. readings appear just as likely to have big babies as those with a high titre. It is unlikely, therefore, that unduly high C.G. readings favour the advanced bony and gonadal maturity in the infant and actually influence the size and weight of the infant, or of the placenta. The fourth tenet of the theory advanced by White was that by prolonged oestrogen therapy during pregnancy a constant depression of the C.G. levels could be maintained. As stated previously, in this discussion, studies on the present series of cases have not confirmed this. It was shown that stilboestrol when given initially to a case invariably produced a fall in the C.G. reading. This depression, however, was but an evanescent one, the reading gradually climbing to its original level when the same dosage was maintained. Gradual increase in dosage as recommended by White produced another transient depression even when the titre had begun to rise but eventually certain patients appeared to become completely refractory to the stilboestrol even when it was given in massive doses, both orally and parenterally, (Cases XI and VI).

The/

The question of whether the administration of stilboestrol sometimes in association with progesterone, is actually of value in lowering the foetal mortality rate in diabetic pregnancies, cannot be evaluated on the small number of cases studied. Many subsequent workers have not confirmed the dramatic results obtained by White using hormonal therapy. In the present study, which extended over a period of approximately fifteen months, the foetal mortality rate in the fifteen cases was 5 or 33.3%. In the previous five years the foetal loss rate in diabetic pregnancies in Edinburgh, reported by Gilbert and Dunlop (1949), was 51.4%. It is however, doubtful if the decrease in foetal mortality obtained is due to the oestrogen therapy per se or to the better clinical and obstetrical care which these patients have received during the last two years. From the purely endocrinological aspect of the problem, it does appear definite that a number of these patients do have abnormally high C.G. readings in comparison with normal women, but little correlation has been observed so far between the C.G. readings on the one hand, and the prognosis of the foetus on the other.

The/

The remainder of the discussion will be devoted to a short review of the clinical aspects of some of these cases.

Insulin requirements during pregnancy in the majority of cases rose as gestation proceeded. This was observed in Cases I, II, IV, VI, VII, VIII, IX, XI, XII, XIII and XV. Case III was exceptional in that the insulin requirements fell during the last trimester. Cases V, X and XIV were treated by dietetic measures alone, no insulin being necessary. These three cases were examples of the older, obese, relatively mild diabetics. The above figures support the contention advanced by Duncan and Fetter (1934), and by Skipper (1933), that carbohydrate tolerance usually diminishes as pregnancy advances. There was no correlation between insulin requirements and C.G. readings.

A striking feature in the series of cases studied has been the virtual absence of pregnancy toxaemia. Workers generally agree that the diabetic is more liable to develop late pregnancy toxaemia than the non-diabetic. In her group of patients showing "hormonal imbalance" (80% of the 261 pregnancies studied), White reported that the incidence of/

of pre-eclampsia was 50% and Barns and Morgan (1949) reported an incidence of 43% in their diabetic pregnancies as compared with an incidence of 35% in non-diabetic pregnancies. In many of their cases, the toxaemia was severe enough to warrant admission to hospital and was of a more severe type than that occurring in non-diabetic pregnancies. Smith and Smith (1944), believe that late pregnancy toxaemia, with its associated hormonal changes, is the cause of many foetal catastrophies in diabetic women. In the fifteen patients studied in Edinburgh, there was no case of full blown pregnancy toxaemia. Case VIII showed slight hypertension at the thirty-second week which persisted until delivery. There was minimal ankle oedema but no albuminuria. A transient albuminuria occurred in Case VII at the thirty-third week associated with some slight oedema of the ankles but there was no hypertension. Case XII had slight ankle oedema at the thirty-fifth week without hypertension and albuminuria. None of the other patients showed any evidence of pregnancy toxaemia. Stilboestrol therapy did not appear to be in any way related to this finding and the reason for the absence/

absence of pregnancy toxaemia was not clear.

Hydramnios was present in four of the fifteen cases. Case III developed a moderate degree of the condition at the thirty-fourth week and a mild degree was present in Cases VI and VII onwards from the thirty-third and thirty-second weeks respectively. Case XI had a severe degree of hydramnios when delivered by Caesarean section at the thirty-sixth week. In this patient, the hydramnios was thought to be due to the massive dosage of oestrogens given.

Nine patients were delivered by Caesarean section, usually of the classical type, and six had an ordinary vaginal delivery usually preceded by a medical induction. There were no maternal deaths in the series. Diabetic coma did not occur in any of the patients during pregnancy. Only in Case I was there a severe hypoglycaemic attack almost amounting to hypoglycaemic coma. In the other patients, these attacks were mild and very easily controlled. In none of the new born infants in whom blood sugars were estimated, was the reading below the normal range for new born infants given by Ketteringham and Austin (1938), and therefore it appears/

appears that neo-natal hypoglycaemia in the infant, like pre-eclampsia in the mother, is not an important cause of the high foetal loss rate in diabetic pregnancies.

C.G. IN MISCELLANEOUS CONDITIONS

(a) Hyperemesis Gravidarum.

Excessive quantities of chorionic gonadotrophin in the blood of cases of hyperemesis gravidarum have been demonstrated by Anselmino and Hoffmann (1936), and Rakoff (1940). Other workers, notably Brindeau, Hinglais and Hinglais (1939), Ebrhardt (1936) and Schoeneck (1942), have noted that the excretion of C.G. in the urine in these cases is also excessive. The experiments of Schoeneck (1942) in this connection are the most noteworthy. This worker had previously noted that in performing routine Friedman tests for the diagnosis of pregnancy some reactions were more intense than others and therefore some urines contained more gonadotrophic hormone than others. The most intense reactions according to Schoeneck occurred in those patients who vomited or were nauseated in early pregnancy. Subsequently, a quantitative Friedman test was evolved based on finding the smallest quantities of urine which would give a positive reaction. It was found that the greater the degree of nausea and vomiting in these patients the smaller/

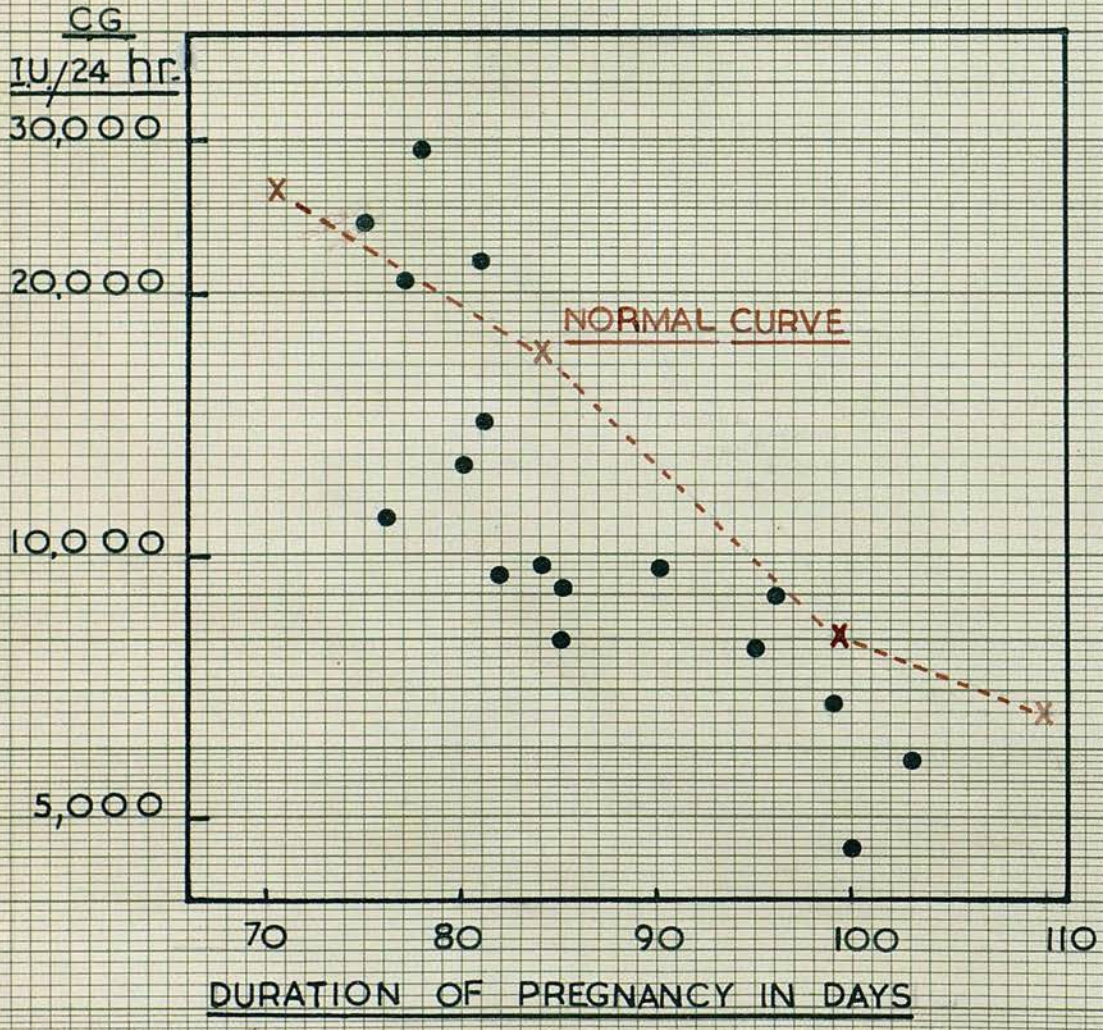

FIG. 22 - ESTIMATIONS OF C.G. IN 7 CASES OF HYPEREMESIS GRAVIDARUM

smaller the quantity of urine required to produce a positive reaction and therefore it was concluded that an excess of gonadotrophic material was found in the urine of patients who, in early pregnancy, vomited or were nauseated. Cases of actual hyperemesis gravidarum gave much higher readings than those with merely a "physiological vomiting of pregnancy." Specimens of urine were obtained from the fifth to fifteenth week of pregnancy.

Only seven cases of true hyperemesis have so far been studied in Edinburgh and altogether seventeen readings have been obtained in these patients (see Fig.22). The method of assay used, again depended on the prostatic weight and results were expressed in I.U. of C.G. per 24-hours. It will be seen that no abnormally high readings were found in these patients and the figures were indistinguishable from those obtained in normal pregnancies.

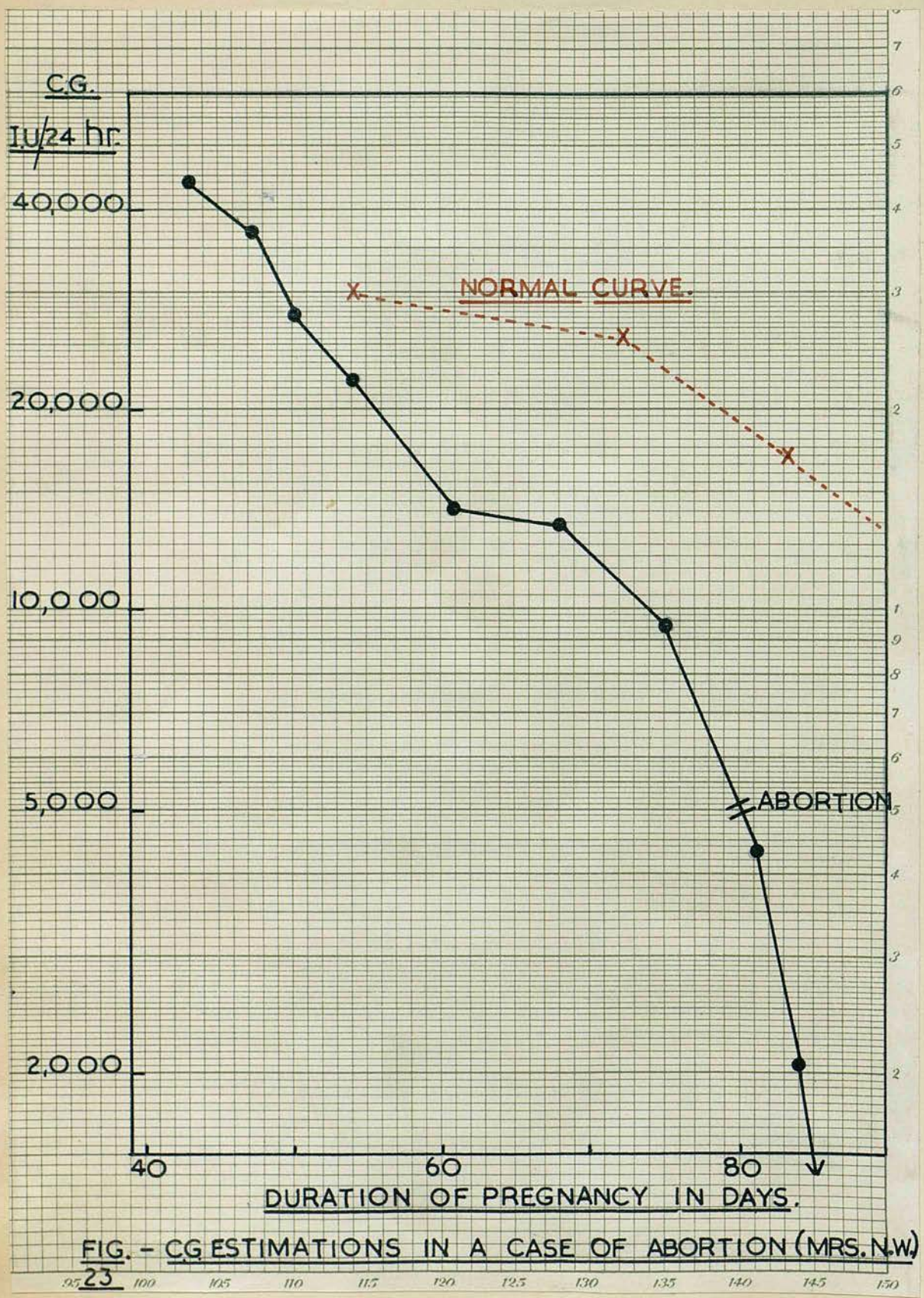

FIG. - C.G ESTIMATIONS IN A CASE OF ABORTION (MRS. N.W.)

(b) Abortion.

Only one case of abortion has so far been studied. The patient, N.W. aged 41, was recommended by the sterility clinic and was first seen on the forty-third day of pregnancy. On the eightieth day an abortion occurred. The results of the C.G. estimations are shown in Fig.23 along with the curve for normal women at a corresponding stage of gestation. It is interesting to note that in the patient studied, the period of upsurge and decline constituting the "peak phase" of excretion of C.G. occurred at an earlier stage of pregnancy than in normal women. By the seventy-fourth day, the reading of C.G. was below the 10,000 I.U. level while in normal women such figures are not usually obtained until about the ninetieth day.

Although no conclusions can be drawn from the small amount of evidence available, it is possible that quantitative estimations of C.G. in cases of habitual abortion might prove of value as an index of the prognosis in such cases.

SUMMARY AND CONCLUSIONS.

(1) A method of biological assay of chorionic gonadotrophin (C.G.) is described. It depends on the increase in the weight of the prostate in immature rats. Results are expressed in terms of the International Standard C.G.

(2) The method depending on the prostatic weight gives more accurate and reliable results than that depending on seminal vesicles.

(3) When the prostatic weight method of assay is employed, preliminary extraction of the urine is unnecessary as the oestrogens in pregnancy urine are not present in sufficient amounts to interfere with the test involving the male organs.

(4) Stilboestrol in dosage of 0.9γ, 9γ and 90γ does not produce any enlargement of the prostate or seminal vesicles in immature male rats.

(5)/

(5) A study of extraction methods for C.G. is presented from the points of view of quantitative recovery and oestrogen removal. Precipitation by acetone or alcohol and the kaolin-acetone-ether method, all give a good quantitative recovery and effectively remove oestrogens as tested by experiments on oöphorectomised animals. The kaolin method without subsequent precipitation by acetone is quantitative, but does not separate the oestrogens from C.G. The efficacy of simple acetone precipitation for routine use is emphasized.

(6) The kaolin-acetone-ether method of extraction, recently developed in this laboratory, is described in detail. The chief advantages of the method are the good recovery of gonadotrophin obtained, the technical simplicity and the ease of concentration. These points should make it an eminently suitable routine method for extracting gonadotrophins from non-pregnant urine.

(7)/

(7) Curves are presented showing the excretion of C.G. at all stages of normal pregnancy. It is pointed out that the variations in C.G. excretion during the later stages of pregnancy are smaller than those reported by previous investigators.

(8) A proportion of pregnant diabetics (six out of the fifteen studied), have an abnormally high titre of C.G. during pregnancy.

(9) Stilboestrol, when administered to normal and to diabetic women initially produces a fall in the C.G. level, but if the same dosage is maintained an "escape" phenomenon occurs. With prolonged dosage of the oestrogen some diabetic patients appear to become completely resistant to stilboestrol.

(10) No correlation can be found between the C.G. readings on the one hand, and the weight of the baby and placenta on the other. Those patients with normal C.G. curves throughout pregnancy seem just as liable to deliver overweight babies as those with abnormally high readings.

(11)/

(11) In no case can it be said that a rise in the C.G. level can be regarded as the herald of an impending obstetrical disaster.

(12) The series of diabetic pregnancies studied is, as yet, too small adequately to assess the effect of stilboestrol in lowering the foetal mortality rate in diabetic pregnancy, but results, so far, do not appear to be very encouraging.

(13) A brief review is given of the medical and obstetric findings in the patients studied. The low incidence of pregnancy toxaemia in the cases considered is noted.

(14) Estimations of C.G. have been obtained in seven cases of hyperemesis gravidarum and the results differ in no way from those obtained in normal pregnancy.

(15) Readings of C.G. have been made in a case of abortion.

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